Forecasting the Vicinal Diketone Content of Finished Beer[1]

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ABSTRACT

Studies were made to calculate the total concentration of acetohydroxy acids (AHA) plus vicinal diketones (VDK) in filtered beer at an early stage of lagering. Concentration of AHA decreased during lagering according to first-order kinetics in which the velocity constant was virtually a function of temperature. Concentration of VDK was determined by integrating the difference in the rates of the following two reactions: 1. Its stoichiometric formation from AHA and; 2. Its absorption by yeasts. Concentration of VDK remained virtually constant from the time, after several days of lagering, when the concentration of yeast cells began to decrease according to first-order kinetics. According to these results, the total concentration of AHA + VDK in filtered beer could be calculated at an early stage of lagering from the concentrations of AHA + VDK and the yeast cells at the day of calculation and the predetermined temperature program.

Key words: *Calculation, Diacetyl, Lagering, Yeast concentration.*

A diacetyl odor sometimes appears in beer although there is no diacetyl odor during lagering. This is because vicinal diketones (VDK)—diacetyl and pentanedione, which have diacetyl odor and are formed by spontaneous oxidative decarboxylation of acetohydroxy acids (AHA)—is absorbed by yeast cells as it is formed in fermenting wort, but remains unabsorbed in the beer after it is filtered (4). Thus, the strength of the diacetyl odor of bottled beer depends on the concentration of AHA (and VDK, if present) in the fermenting wort subjected to filtration. On the basis of this mechanism of VDK formation, the three following methods should be effective for obtaining beer with no diacetyl odor after a short period of lagering: 1. Suppression of AHA formation by control of amino acid absorption during fermentation (7), 2. acceleration of AHA decomposition by raising the temperature (1,3,5,9–11,14) or lowering the pH value (2,5,12,15,16) during lagering, and 3. keeping enough yeast cells in suspension to absorb VDK which is formed by decomposition of AHA. With regard to the third point, the quantitative relation between the concentration of yeast cells in suspension and the concentration of unabsorbed VDK in fermenting wort was studied, and a simple method for calculating the AHA + VDK concentration in filtered beer at an early stage of lagering was developed.

MATERIALS AND METHODS

Traditional-type bottom fermentations in the Takasaki brewery of the Kirin Brewery Co., Ltd. were analyzed. Wort of 11°P, made from a mixture of barley malt and adjuncts (30%), was fermented in a cubic tank for 1 week at a maximum temperature of 8°C and transferred to a lagering tank at a time when about 90% of the fermentable sugars was exhausted. Lagering started at 3.5°C in a horizontal cylindrical tank and the temperature decreased gradually to a final temperature of −1°C.

The number of yeast cells in suspension was measured with a hemocytometer. AHA and VDK were determined by the method proposed in 1970 (5), and expressed as mg/l. of diacetyl.

RESULTS

With increase in the number of yeast cells in suspension in fermenting wort during lagering, the concentration of unabsorbed VDK in the wort decreases but wort filtration becomes more difficult. Thus, to facilitate filtration a low concentration of yeast is desirable. Accordingly, the quantitative relation between the yeast concentration and the VDK concentration in fermenting wort was studied.

When yeast cells stop growing AHA is no longer formed and it decomposes stoichiometrically to VDK at a rate dependent upon the temperature (θ in °C) and pH of the wort. Thus the rate of VDK formation during lagering, which is equal to the rate of AHA decomposition, is expressed by the following equation:

$$\frac{d\,(VDK)}{dt} = k_{AHA}\,(AHA)_t \qquad (1)$$

where $(AHA)_t$ is the concentration of AHA after t days of lagering and k_{AHA} is a constant (in day^{-1}) for decomposition of AHA calculated by the equation:

$$\log k_{AHA} = 0.0602\,\theta - 1.222 \qquad (2)$$

when the reaction proceeds in our fermenting wort (pH 4.2) (6). In fermenting wort containing yeast cells the VDK formed is absorbed by the cells and converted to odorless acyloins and then diols. The absorption follows first-order kinetics (8) and the rate is expressed by the following equation:

$$\frac{d(VDK)}{dt} = -k_{VDK}\,(VDK)_t \qquad (3)$$

where $(VDK)_t$ is the concentration of VDK after t days of lagering and k_{VDK} is a function of the temperature (θ in °C) and yeast concentration (Y in cells/ml). The k_{VDK} values for various yeast strains at 2°C are very similar, as shown in Table I. Changes in the k_{VDK} with change in temperature are shown in Fig. 1. With increase in temperature the rate of increase of k_{VDK} was less than that of k_{AHA} ($Q_{10} = 3.5$).

Change in the VDK concentration was calculated on the basis of these findings. The VDK concentration after t days of lagering is

$$(VDK)_t = \int_0^t [k_{AHA}\,(AHA)_t - k_{VDK}\,(VDK)_t]dt$$
$$= \frac{k_{AHA}}{k_{VDK} - k_{AHA}}\,(AHA)_0\left[e^{-k_{AHA}\cdot t} - e^{-k_{VDK}\cdot t}\right] + (VDK)_0\,e^{-k_{VDK}\cdot t} \qquad (4)$$

where $(AHA)_0$ and $(VDK)_0$ are the concentrations of AHA and VDK at start of lagering, respectively. As the temperature and yeast

TABLE I
Rates of Vicinal Diketone Absorption by Various Yeast Strains

Yeast Strain	VDK Absorption-Rate Constant $day^{-1} \cdot 10^{-6}$ ml/cell, at 2°C
Brewer's yeast, bottom A	0.26
Brewer's yeast, bottom B	0.18
Brewer's yeast, bottom C	0.27
Brewer's yeast, top (NCYC 240)	0.26
Baker's yeast	0.23
Wine yeast (OC 2)	0.17

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concentration vary day-by-day during lagering, this calculation was made by computer once a day using k_{AHA} and k_{VDK} values calculated from the means of the temperatures and yeast concentrations on that day and the previous day. As shown in Fig. 2 calculated values agreed well with measured ones.

These results stimulated the development of a method for calculating the total concentration of AHA + VDK in filtered beer at an early stage of lagering. A similar study had been made by Rice *et al.* in 1973 (13). However, yeast concentration changes so markedly under traditional lagering conditions that a significant concentration of VDK, as measured in fermenting wort at the end of lagering and yeast concentration, is mainly determined not by the temperature during lagering but by the flocculating character of the yeast strain used. Thus, the change in its concentration should be taken into account for calculation of the AHA + VDK concentration in filtered beer. For this purpose it was necessary to forecast changes in temperature and yeast concentration. Change in temperature can be estimated easily, because it is controlled according to a predetermined program.

$$\theta_t = \theta_c - \frac{4.5}{T}(t - t_c)$$

where θ_t and θ_c are the temperatures (in °C) after t days of lagering and on the day of the calculation (t_c), respectively. T is the time (in days) required for cooling from the temperature at start of lagering (3.5°C) to the temperature at filtration (−1°C). As the lowest temperature during lagering is −1°C, θ_t does not decrease below −1°C. A semilogarithmic plot of the change in yeast concentration with time showed that the yeast concentration decreases according to first-order kinetics, except for the first few days of lagering, and the rate constant of decrease was about 0.07 day^{-1} in most fermentations.

$$Y_t = Y_c\, e^{-0.07\,(t-t_c)}$$

where Y_t and Y_c are the concentrations of yeast cells after t days of lagering and on the day of the calculation (t_c), respectively, and t_c should be later than the 5th day.

It seemed possible to calculate the AHA + VDK concentration in beer at the time of filtration from measurements taken several days after the beginning of lagering, but it was found desirable to know the individual concentrations of AHA and VDK on the day of calculation. These can be determined by the method proposed in

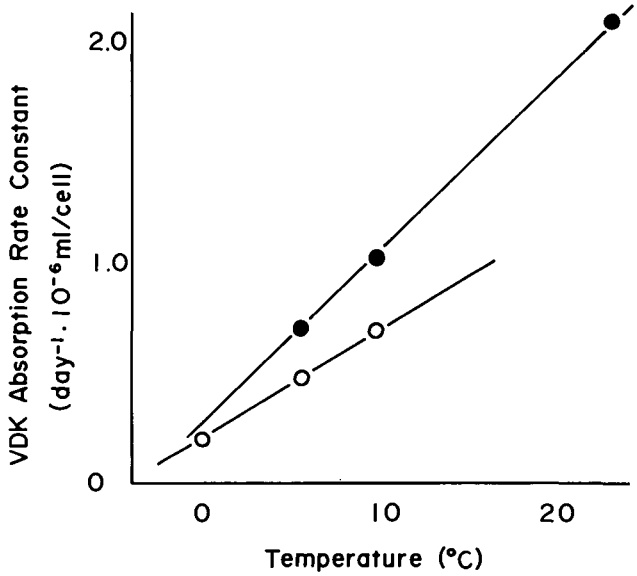

Fig. 1. Change in vicinal diketone absorption-rate constant with change in temperature; o = diacetyl, • = pentanedione.

TABLE II
Change in Vicinal Diketone Concentration under Various Lagering Conditions[a]

Lagering Time in Days	AHA mg/l. as DA	VDK (mg/l. as DA) 0.05[b]	0.06[b]	0.07[b]	0.08[b]
10	0.19	0.05	0.05	0.04	0.04
20	0.11	0.06	0.05	0.04	0.03
30	0.06	0.07	0.05	0.04	0.03
40	0.04	0.08	0.06	0.05	0.04
50	0.02	0.08	0.07	0.05	0.04

[a]AHA concentration at the beginning of lagering = 0.4 mg, calculated as diacetyl (DA), per liter. Time required for cooling the temperature from 3.5°C to −1°C = 15 days.
[b]Rate constant for decrease in yeast concentration (day^{-1}).

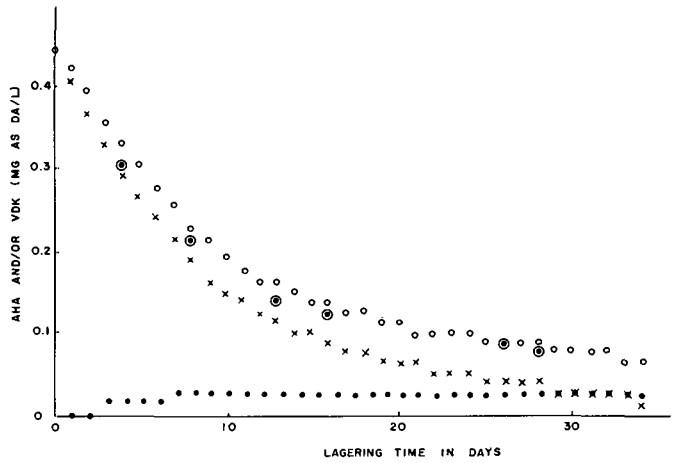

Fig. 2. Comparison of measured and calculated concentrations of AHA + VDK; • = VDK calculated, x = AHA calculated, o = AHA + VDK calculated, o = AHA + VDK measured. (Ordinate is in mg, calculated as diacetyl (DA) per liter).

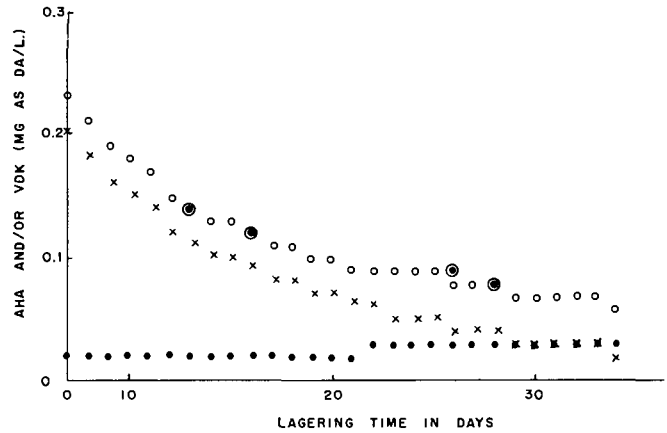

Fig. 3. Comparison of measured concentrations of AHA + VDK with values calculated from the AHA + VDK concentration and the yeast concentration on the 7th day of lagering • = VDK calculated, x = AHA calculated, o = AHA + VDK calculated, ⊙ = AHA + VDK measured. (Ordinate is in mg, calculated as diacetyl (DA) per liter).

TABLE III
Change in AHA Concentration under Various Temperature Programs

Lagering in Days	Tc = 10[a]	Tc = 15[a]	Tc = 20[a]	Tc = 25[a]	Lagering in Days	Tc = 10[a]	Tc = 15[a]	Tc = 20[a]	Tc = 25[a]
0	0.400	0.400	0.400	0.400					
1	0.366	0.365	0.365	0.364	26	0.088	0.079	0.072	0.065
2	0.337	0.334	0.333	0.332	27	0.083	0.075	0.069	0.061
3	0.311	0.307	0.305	0.304	28	0.079	0.071	0.065	0.058
4	0.289	0.283	0.281	0.279	29	0.075	0.068	0.062	0.055
5	0.270	0.262	0.259	0.256	30	0.071	0.064	0.059	0.053
6	0.253	0.243	0.239	0.236	31	0.067	0.061	0.056	0.050
7	0.238	0.227	0.221	0.217	32	0.064	0.058	0.053	0.047
8	0.225	0.212	0.205	0.201	33	0.061	0.055	0.050	0.045
9	0.213	0.198	0.191	0.186	34	0.058	0.052	0.048	0.043
10	0.202	0.186	0.178	0.172	35	0.055	0.050	0.045	0.040
11	0.192	0.175	0.167	0.162	36	0.052	0.047	0.043	0.038
12	0.182	0.165	0.156	0.149	37	0.049	0.045	0.041	0.036
13	0.173	0.156	0.146	0.139	38	0.047	0.042	0.039	0.035
14	0.164	0.148	0.138	0.130	39	0.044	0.040	0.037	0.033
15	0.156	0.141	0.130	0.122	40	0.042	0.038	0.035	0.031
16	0.148	0.134	0.123	0.114	41	0.040	0.036	0.033	0.030
17	0.140	0.127	0.116	0.107	42	0.038	0.034	0.031	0.028
18	0.133	0.120	0.110	0.101	43	0.036	0.033	0.030	0.027
19	0.126	0.114	0.104	0.095	44	0.034	0.031	0.028	0.025
20	0.120	0.108	0.099	0.090	45	0.032	0.029	0.027	0.024
21	0.114	0.103	0.094	0.085	46	0.031	0.028	0.025	0.023
22	0.108	0.098	0.089	0.080	47	0.029	0.026	0.024	0.022
23	0.102	0.093	0.085	0.076	48	0.028	0.025	0.023	0.021
24	0.097	0.088	0.080	0.072	49	0.026	0.024	0.022	0.019
25	0.092	0.084	0.076	0.068	50	0.025	0.023	0.021	0.019

[a]Tc = Time required for cooling from 3.5°C to −1°C (day).

TABLE IV
Method for Forecasting VDK + AHA Concentration in Filtered Beer

1. The concentrations of AHA and VDK on the day of forecasting are calculated from the total concentration of AHA + VDK and that of yeast cells by equations (7) and (8). The concentration of VDK on the day of the calculation is equal to that on the day of filtration.

2. By using a table listing change in unit concentration of AHA in the given temperature program, the concentration of AHA on the day of filtration is calculated from that on the day of the calculation.

3. The sum of the concentrations of AHA and VDK on the day of filtration is calculated.

[a]Time required for cooling from 3.5°C to −1°C = 20 days, AHA + VDK on filtration = 0.15 mg/l. as DA.
[b]Lagering period.

TABLE V
Examples of Forecasts

Day of Calculation	Day of Filtration	Calculated Concentration of AHA + VDK mg/l. as DA	Measured Concentration of AHA + VDK mg/l. as DA
6	26	0.06	0.06
6	26	0.06	0.06
9	19	0.11	0.11
9	19	0.14	0.14
8	30	0.07	0.06
8	30	0.05	0.04

1970 (5) or by other methods, but these methods are time-consuming and not suitable for routine use. It was found that

$$\frac{(AHA)}{(VDK)} = \frac{k_{VDK}}{k_{AHA}} \tag{5}$$

when k_{VDK} is sufficiently larger than k_{AHA}. It was calculated from the results in Fig. 2 that

$$\frac{k_{VDK}}{k_{AHA}} = 3.3 \times 10^{-6} Y \tag{6}$$

at the temperatures observed during lagering (3.5°C to −1°C). Thus the concentrations of AHA and VDK after c days of lagering were calculated by the following equations:

$$(AHA)_c = \frac{3.3 \times 10^{-6} Y_c}{1 + 3.3 \times 10^{-6} Y_c} (AHA + VDK)_c \tag{7}$$

$$(VDK)_c = \frac{1}{1 + 3.3 \times 10^{-6} Y_c} (AHA + VDK)_c \tag{8}$$

where $(AHA + VDK)_c$ is the total concentration of AHA + VDK, which is easily determined by measuring VDK concentration after incubating the sample at 60°C for 90 min (5). Thus, a method was developed to calculate the total concentration of AHA + VDK that would be present in filtered beer at an early stage of lagering. The results of calculations by this method are shown in Fig. 3. Again there was good agreement between calculated and measured values.

It was also found that VDK concentration varied only slightly during lagering, as shown in Table II. This suggested that the method for calculation could be greatly simplified. That is, the

TABLE VI
Relation between Lagering Period and Appropriate Concentration of Yeast Cells in Suspension[a]

AHA Concentration at the Beginning of Lagering mg/l. as DA	Appropriate Concentration of Yeast Cells on the 10th Day of Lagering ($\times 10^6$ cells/ml)		
	20 days[b]	30 days[b]	40 days[b]
0.4	1.0	0.6	0.5
0.6	40	1.3	0.8
0.8		3.3	1.3
1.0		45	2.1

concentration of AHA + VDK in filtered beer could be calculated by adding the concentration of VDK on the day of the calculation to the concentration of AHA at the time of filtration. Further, the change in AHA concentration could be estimated without a computer from a table of changes in unit concentration of AHA under various temperature programs (Table III). The simplified method developed for forecasting the total concentration of AHA + VDK in filtered beer is shown in Table IV. Table V shows the results of several forecasts.

DISCUSSION

If the period of lagering is long, it is not necessary to have many yeast cells in suspension in the fermenting wort, because most of the AHA decomposes during the long period of lagering; thus, the presence of some VDK will not enhance the diacetyl odor of the beer. However, when the lagering period is short, a high concentration of yeast cells in suspension is necessary to prevent the accumulation of VDK in the fermenting wort. Thus, the appropriate concentration of yeast cells in suspension depends upon the age of the beer. When the threshold value for VDK is 0.15 mg/l. as diacetyl, the relation between the age of beer and the appropriate concentration of yeast cells in suspension can be calculated as shown in Table VI on the basis of the findings in this report.

The relation of accumulation of VDK to the age of the beer and the concentration of yeasts in suspension can be elucidated from the present findings, but it is still uncertain how to control the concentration of yeast during lagering. Kräusening is known to be very effective for keeping yeasts in suspension. The presence of some fermentable sugars at the end of primary fermentation is also said to be effective for this purpose. However, further quantitative studies are required on the effects of these factors.

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