A Quality Control Method for the Determination of Vicinal Diketones and Precursors in Fermenting Wort[1]

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ABSTRACT

A method is proposed for the determination of vicinal diketones (VDK) and precursors in fermenting wort. The method, which can be used for routine quality control analysis, is an improvement over the previously published ASBC method in that the conversion of precursors to VDK is done during the sweeping of the sample with oxygen at 75° C, thus eliminating an additional sample treatment step to convert precursors to VDK. The equipment used is easy to construct, low in cost, and permits the assembly of several apparatuses for simultaneous determinations. The proposed method possesses several advantages over other published methods, eg, fewer interferences, higher sensitivity, and complete conversion of precursors to VDK. More important the results obtained are very similar to those obtained by a gas chromatographic method.

Key words: *Diacetyl, Fermenting wort, Quality control, Spectrophotometric method, Total vicinal diketones*

In recent years there has been a tendency for the construction of large-capacity fermenters, resulting in optimization of space and plant capacity and in a more uniform beer (15).

Modern fermentation systems use large-capacity fermenters, which can be used as fermentation tanks, storage tanks, and as a combined tank (19).

In these systems, total vicinal diketones (VDK) content (diacetyl, pentanedione, and their precursors) is frequently used as a criterion to determine the end of fermentation (12).

Large-capacity fermenters have been installed in three of our breweries; to make optimum use of these tanks, an analytical method that could give the total VDK content of the fermenting wort was needed.

A method to be used in routine quality control should have several characteristics such as: ability to provide complete conversion of precursors to VDK, few interferences, low cost and readily available equipment, short analysis time, high sensitivity, and reproducible results.

We recently described (7) a spectrophotometric method for the quantitative determination of total VDK. The sample treatment step includes heating the sample with stirring at 60° C for 75 min at a pH of 4.1 ± 0.1 in an oxygen atmosphere to achieve complete conversion of precursors to VDK. However, because the method is lengthy, laborious, and thus not appropriate for routine analysis, we attempted to improve the number of operations to reduce the time of analysis.

In 1978, Inoue (8) reported innovations to the micro method (14) that reduced the volatilization time of the sample from 120 to 30 min by using a volatilization temperature of 90 instead of 65° C, without affecting the efficiency of the extraction of VDK.

The above innovations to the micro method and our results on the optimum conditions to convert precursors to VDK suggested the possibility of using oxygen instead of nitrogen during the sweeping of the sample; this would convert precursors to VDK during that step, eliminating an additional sample treatment step.

The resulting procedure, called the Improved Total VDK-Ultraviolet (UV)-Micro, was applied to fermenting wort and compared with other published methods. The results are presented in this article.

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EXPERIMENTAL

All the compounds used in the evaluation of the methods were purified by preparative gas chromatography. Several modifications of our previously published method (7) were made. 1) The concentration of the hydroxylamine solution was 8%, w/v. 2) The sample treatment step was eliminated. 3) The separation step was carried out under the following conditions: sample—cold carbonated beer or cold fermenting wort free of yeast; volatilization apparatus—connected in series (8); volatilization temperature—75 ± 2.5° C; carrier gas—oxygen; and volatilization time—60 min. 4) The two changes to the detection step were that the volume of the distillate was made up to 10 ml before evaporation, and evaporation was a volume of 4 ml, using a heating plate for 5–10 min.

The equipment used (Fig. 1) was originally designed by Rice et al (16).

The resulting Improved Total VDK-UV-Micro method was compared with the following methods: the European Brewery Convention (EBC) method (*o*-phenylenediamine) (5), the Institute of Brewing (IoB) method (α-naphthol-creatine) (11), the ASBC-UV method (hydroxylamine) (1), and the Improved-Micro method (8,10).

The first three methods were designed for finished beer. Therefore, to apply them to fermenting wort, the samples had to be subjected to a sample treatment step to convert precursors to VDK. The treatment was digestion with stirring at 60° C for 75 min at a pH of 4.1 ± 0.1 in an oxygen atmosphere.

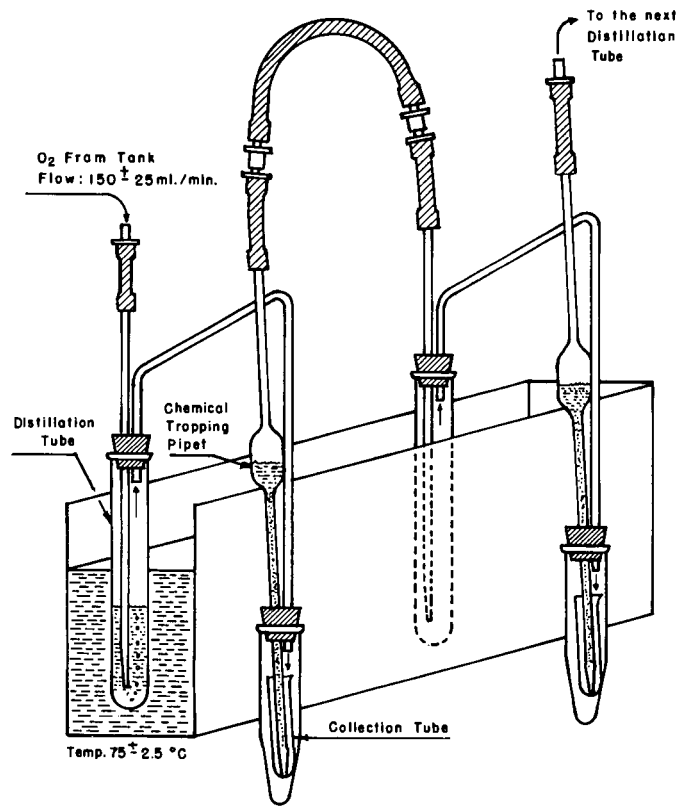

Fig. 1. Volitization apparatus (from Rice et al [16]).

The experimental procedure described for each method was followed, with certain modifications. In the EBC and IoB methods, the distillation apparatus used to determine ethanol in beer (2) was used. In the ASBC-UV method, the following changes were made: 1) the distillate was collected in 2 ml of distilled water and 1 ml of the hydroxylamine solution; 2) all the distillate was digested (80° C, 15 min) and evaporated to a volume of 6–7 ml; and 3) the blank was prepared in the same way as the blank used to construct the calibration curve.

A gas chromatographic method (7) was used as a reference method.

RESULTS AND DISCUSSION

Sensitivity

The sensitivity of each method to the different compounds tested is presented in Table I. The ASBC-UV, the improved micro, and the proposed method have higher sensitivity to diacetyl and 2,3-pentanedione. The table also shows which compounds interfere with each method.

TABLE I
Sensitivity[a] of the Methods

	Method				
Compound	EBC[b]	IoB[b]	ASBC-UV[b]	Improved Micro[b]	Proposed
Diacetyl	0.260	0.376	1.092	0.912	0.893
2,3-Pentanedione	0.131	0.108	0.635	0.533	0.688
Methylglyoxal	0.011	n.d.	0.039	0.0009	n.d.[c]
Acetoin	n.r.[d]	0.008	n.d.	n.r.	n.d.
Acetaldehyde	n.d.	n.d.	0.004	n.r.	0.002

[a] Slope of the calibration curve obtained by adding standards to beer and subjecting the mixture to the procedure described in each method.
[b] Methods described in references 5, 11, 1, and 8 and 10, respectively.
[c] Not detected.
[d] No reaction.

TABLE II
Comparison of Methods: Lowest Detectable Concentration of Diacetyl, Reproducibility, and Length of Analysis Time

Method[a]	Lowest Diacetyl Concentration Detectable[b] (mg/L)	Coefficient of Variation[c] (%)	Analysis Time per Sample[d] (min)
EBC	0.019	± 4.43	135
IoB	0.013	± 2.38	111
ASBC-UV	0.005	± 6.24	155
Improved micro	0.005	± 3.90	100
Proposed	0.005	± 6.40	95

[a] The first four methods are described in references 5, 11, 1, and 8 and 10, respectively.
[b] Calculated from the data in Table 1, assuming the photometric accuracy of a common spectrophotometer.
[c] Obtained from 10 analyses of a sample of fermenting wort with approximately 0.15 mg/L of total vicinal diketones (as diacetyl).
[d] Methods 1–3 include 90 min of sample preparation.

The sensitivity of the method is an important characteristic because if the method is to be used to monitor the progress of the fermentation it must be sufficiently sensitive to detect variations in total VDK of 0.01 mg/L or lower when using a common spectrophotometer with a photometric accuracy of 0.005 absorbance units.

Another way of representing the sensitivity of a method is to determine the lowest detectable concentration of a compound. Table II shows the lowest detectable concentration of diacetyl by each of the methods evaluated. The values were calculated from the data in Table I, assuming the photometric accuracy of a common spectrophotometer.

Interferences

The compounds tested as possible interferences in the determination of diacetyl were 2,3-pentanedione, methylglyoxal, acetoin, and acetaldehyde. The degree of interference in the methods by each of the compounds tested is shown in Table III and is expressed as the contribution of each compound to the total absorbance reading.

To calculate the numbers reported in Table III, we used the values from Table I and the average concentration in beer of the different compounds tested (17,18,20).

Considering the difficulty of determining diacetyl alone without also determining 2,3-pentanedione, one can conclude from the data in Table III that the improved micro and the proposed method present a lower degree of interference; approximately 90% of the absorbance corresponds to VDK (diacetyl and 2,3-pentanedione).

The conclusions drawn from Table III are in agreement with the reports for 1979 and 1980 of the ASBC Subcommittee on Vicinal Diketones and Precursors, which collaboratively tested the EBC and IoB methods; high results obtained with the IoB method were attributed to the interference of acetoin (4). Also, high results were obtained with the EBC method when compared to other methods (3).

Reproducibility

An analytical characteristic of a method that is affected a great deal by its sensitivity is its reproducibility. The more sensitive a method is, the more likely it is to be affected by any change in the extraction procedure or any other change during the course of the analysis. Table II shows the reproducibility of the different methods evaluated.

Apparatus and Analysis Time

The two factors that most influence the economic aspects of an analytical method are how expensive the equipment is and how fast the results are obtained.

Among the five methods tested, the proposed method uses equipment that is easiest to construct and lowest in cost, and it also permits the assembly of several pieces of apparatus for simultaneous determinations.

Recently, Inoue reported four types of apparatus with different

TABLE III
Percent Contribution of the Compounds Tested to the Total Absorbance Reading in Each Method[a]

	Average Concentration in Beer	Method				
Compound	(mg/L)	EBC[b]	IoB[b]	ASBC-UV[b]	Improved Micro[b]	Proposed
Diacetyl	0.08	41.18	57.69	39.73	59.35	59.66
2,3-Pentanedione	0.06	15.68	11.54	17.35	26.02	33.61
Methylglyoxal	2.00	43.14	···	35.62	14.63	···
Acetoin	2.00	···	30.77	···	···	···
Acetaldehyde	4.00	···	···	7.30	···	6.73

[a] Values were calculated from the concentrations and the data of Table I.
[b] Methods described in references 5, 11, 1, and 8 and 10, respectively.

TABLE IV
Comparative Analysis of Fermenting Wort for Vicinal Diketones (mg/L) by the Original[a] and Proposed Methods

Sample	Method	
	Original	Proposed
1	0.19	0.18
2	0.29	0.20
3	0.42	0.42
4	0.27	0.30
5	0.14	0.13
6	0.12	0.14
7	0.15	0.13
8	0.12	0.16

[a] Reference 7.

TABLE V
Determination of Total Vicinal Diketones in Fermenting Wort

Method[a]	Concentration[b] (mg/L)
Gas chromatography	0.23[c]
EBC	0.48
IoB	0.34
ASBC UV	0.28
Improved micro	0.13
Proposed	0.19

[a] Methods 2–5 are described in references 5, 11, 1, and 8 and 10, respectively.
[b] Average of 10 samples of fermenting wort (6th and 7th day).
[c] Composed of 0.14 mg/L diacetyl and 0.09 mg/L 2,3-pentanedione.

degrees of sophistication to be used with the improved micro method (8,9), resulting in a shorter analysis time per sample; however, that equipment is more expensive and complicated than the equipment used in the proposed method.

With respect to time of analysis per sample (Table II), in the EBC, IoB, and ASBC-UV methods the sample is first subjected to a treatment to convert precursors to VDK (7), increasing the time of analysis to 90 min, and in the improved micro method the sample is heated at 90°C for 10 min before the analysis (8).

The proposed method does not require a conversion step because, during the volatilization of the sample, a complete conversion of precursors to VDK is obtained. The fact was corroborated by analyzing several samples of fermenting wort by our previously published method (7) and by the proposed method (Table IV).

Eliminating the conversion step and following the experimental procedure described above gave a reduction of analysis time from 270 to 95 min, a 65% reduction in time, resulting in an analysis taking 35% as much time as the original.

Analysis of Fermenting Wort

Ten samples of fermenting wort were analyzed by each of the five methods evaluated and by the gas chromatographic method used as reference; the results are shown in Table V.

The results obtained with the Improved Total VDK-UV-Micro method correlate very well with those obtained by the gas chromatographic method. The lower results obtained with the improved micro method are due to incomplete conversion of precursors to VDK; when the fermenting wort to be analyzed for total VDK was subjected to the sample treatment step (7) and then analyzed by the improved micro method, the results obtained were similar to those obtained by the proposed method.

The lower results obtained by the improved micro and the proposed methods are due to the fact that diacetyl is used in the construction of the calibration curves and this compound gives a greater response than the 2,3-pentanedione that is determined simultaneously (Table I) (6,13).

CONCLUSION

An Improved Total VDK-UV-Micro method which can be used in routine quality control, is proposed for the determination of total VDK in fermenting wort.

The method possesses several advantages over other published methods such as fewer interferences, high sensitivity, complete conversion of precursors to VDK, short time of analysis, acceptable reproducibility, and low-priced readily available equipment.

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