Revised Methods of Diacetyl Analysis

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Keywords: Broad spectrum, Distillation, Gas chromatography, Micro, Precursor conversion, Total VDK, UV spectrophotometer

CONCLUSIONS

1. The Broad Spectrum, Micro, and Gas Chromatographic methods for the analysis of diacetyl were the three most frequently used procedures, and the UV Spectrophotometer Method was least used.
2. The Reference (Distillation) Method was used by few laboratories and with some reservations by those who did use this procedure.
3. There was unanimous agreement that the Gas Chromatographic Method, although modified by a substantial number of users, measured diacetyl and 2,3-pentanedione separately, and that the levels of these compounds depend on sample treatment.
4. The effect of sample treatment was a concern common to all methods because sample treatment affects precursor conversion to diacetyl and 2,3-pentanedione.

RECOMMENDATIONS

1. Rewrite the introduction to Diacetyl methods in *Methods of Analysis*, as given in the Appendix, to clarify the specificity of each procedure and to stress the importance of sample treatment to control the impact of precursors on the results obtained.
2. Remove the UV Spectrophotometer Method (Beer-25D) from the next edition of *Methods of Analysis* and place it in the archives.
3. Encourage the membership to develop standard procedures for the pretreatment of samples.
4. Continue subcommittee studies to determine the specificity of each method, except the Gas Chromatographic Method, for analysis of diacetyl as given in *Methods of Analysis*.

This subcommittee was charged with two tasks: 1) to poll the membership to determine what methods are being used for the analysis of diacetyl or vicinal diketones (VDK), including precursor conversion, and 2) to assess the specificity of each of the five ASBC methods (1).

PROCEDURE

1. A questionnaire was sent to the membership. The responses were compiled to determine the number of laboratories that are using each of the five methods. Method modifications and general comments were recorded, and agreement or disagreement with the stated specificity of each method was noted. Literature references or copies of procedures for non-ASBC methods were collected from respondents for consideration by the Coordination of New and Alternate Methods of Analysis Subcommittee should additional study of diacetyl methods be desirable. In the letter accompanying the questionnaire, members were asked to provide details of the procedures they used for assessing VDK precursors, and these responses were summarized.
2. Subcommittee collaborators were asked to investigate by any means available the specificity of methods currently in use and of other procedures if possible.

RESULTS AND DISCUSSION

Survey Results

The 68 replies to the questionnaire (Table I) reported procedures being used at 82 worldwide locations. Several companies reported the use of two or three of the methods, while six were using non-ASBC procedures.

Methods Evaluation

The Gas Chromatographic Method (Beer-25E) was used more than any other method. The method was modified as published by approximately half of those using this procedure. Most modifications resulted from the use of automated headspace analyzers and involved different columns and/or packings, changes in sample size, different internal standards or the use of an external standard or standard addition calibration curve, and different incubation temperatures and times. Users agreed unanimously that this method determined diacetyl and 2,3-pentanedione separately. Sample handling, including autosampler incubation time and temperature, controlled whether "as-is", partly converted, or total VDK was measured.

The Micro Method (Beer-25C) was the second most frequently used procedure. It was modified by one third of the users, and in most instances the changes were related to the apparatus. The introduction to the diacetyl procedures in *Methods of Analysis* states that this method is specific for diacetyl. Most users disagreed and either used sample treatment to ensure precursor conversion for a total VDK result or calculated a correction factor. Many of the comments concerned the partial or total conversion of precursors during analysis. The method was considered cumbersome, requiring training and experience to obtain reproducibility, and unsuitable for low levels of VDK. On the other hand, it was reported as excellent for simultaneous routine analysis of several samples.

The Broad Spectrum Method (Beer-25B) was the third most frequently used method, and half the users modified the procedure. Different sample and/or reagent volumes and the time between the last reagent addition and the color reading were the main changes. The nonspecificity of this method appeared to be recognized by most users. Response was mixed as to what it determined: half the users agreed it measured as-is VDK, half reported it determined total VDK. There was concern about the color reaction, that is, rapid fading beyond the specified development time and interference by acetoin, especially at higher levels of acetoin. Other concerns were poor reproducibility and partial but not complete precursor conversion during sample preparation.

The Reference Method (Beer-25A) was used by about 15%

TABLE I
Tabulation of Questionnaire Responses

Method	Number of Laboratories Using Method	
	Without Modification	With Modification
A Reference	9	1
B Broad Spectrum	14	7
C Micro	23	7
D UV Spectrophotometer	5	2
E Gas Chromatographic	30	20
Non-ASBC	6	...

of those responding. No modification was reported, and most users agreed that the procedure determined diacetyl as stated. Those who disagreed usually reported the results as total VDK, and there was speculation on the probable conversion of precursors during analysis.

The UV Spectrophotometer Method (Beer-25D) was used least and without modification. The published procedure implies that the method is specific for diacetyl. Users disagreed and reported the results as total VDK. This was reflected in the comments, which were primarily concerned with precursor conversion.

Precursor Conversion Methods

Methods for converting VDK precursors were provided in 12 responses. All procedures were basically the same, namely, aeration and heat. Aeration was achieved by either direct addition or incorporation of headspace air. The temperatures used were 60, 65, or 100°C. These were provided by water bath, incubator, autosampler, or the gas chromatography oven, with heating times varying from 10 min to 2 hr. Time-temperature combinations were arbitrary. One laboratory had investigated conversion by $CuSO_4$, but no details were given.

Specificity Studies

From experience with both the Distillation and Gas Chromatographic methods, one collaborator concluded that all methods probably produce increasing amounts of diacetyl because of precursor conversion, with the degree of increase depending on the times and temperature involved in analysis. No detailed studies were performed for specificity of the methods. Further study of the mechanics of diacetyl formation and analysis methods was considered desirable.

Another collaborator found that the Gas Chromatographic Method, modified to autoanalyzer requirements, detected diacetyl and 2,3-pentanedione separately. However, when VDK precursors were high, there was partial conversion during autosampler equilibration, giving false high readings for diacetyl. This was demonstrated by subjecting a fermentation sample with high precursors to different equilibration times, resulting in increased levels of diacetyl and 2,3-pentanedione with longer heating times.

This same collaborator also found that the Broad Spectrum Method produced results closer to free VDK than to total VDK. Acetoin, if present at high levels, distilled over raising the reading for VDK. Distillates analyzed by gas chromatography were found to contain acetoin. The lack of specificity made the method well suited to operating control since all the compounds producing response in this procedure were deleterious to beer flavor.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis,* 7th ed. Beer-25 Diacetyl. The Society: St. Paul, MN, 1979.

APPENDIX

DIACETYL

Determine diacetyl content of beer by one of the following methods.

The Gas Chromatographic Method (Beer-25E, International Method) is specific for diacetyl; it measures diacetyl and 2,3-pentanedione separately. Other methods determine vicinal diketones (VDK). All methods are affected by sample treatment before and during analysis; VDK precursors will convert to free compounds, partially or completely, depending on the pH, extent of exposure to air, and temperature.

The UV Spectrophotometer Method is available from archives.