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Analytical Methods

Determination and quantitative analysis of acetoin in beer with headspace sampling-gas chromatography

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ABSTRACT

A new method was developed for sensitive determination and quantitative analysis of acetoin in beer in our laboratory. Identification of derivative of acetoin (diacetyl) was carried out by headspace sampling-gas chromatography (HS-GC). The optimal equilibrium time of diacetyl was 32 min for the mixture of 4.5 mL iron reagent and 0.5 mL beer with the stirring of a shaker. As compared to 3 level calibrations, data obtained by 6 level calibrations were more accurate and consistent, and its correlation coefficient was between 0.99 and 0.999. The measuring precision for diacetyl was significantly improved to $\leqslant$2% R.S.D (relative standard deviation) under the condition of HS (30 psi)/GC (22 psi) when compared to HS (30 psi)/GC (25 psi). Detecting precision of two level temperatures was $\leqslant$1.5% R.S.D, which made all the detecting values existing between UCL (upper control limit) and LCL (lower control limit) in comparison with one level configuration. It was more reliable and suitable for the analysis of diacetyl to set up temperature configuration of two levels in HS-GC. Our results suggested that this method could be used successfully to analyse the concentration of acetoin in beer.

1. Introduction

Acetoin is a natural by-product of fermentation process, its determination and quantitative analysis assists in the evaluation of complete and proper fermentation (Brandolini et al., 1995). Improper fermentation results in reprocessing or dumping of the beer (Gorinstein et al., 1999). In recent years, gas chromatography (GC) with electron capture detector (ECD) has gained popularity because it is a sensitive and selective detection technique for electron-active groups (Casella & Contursi, 2003). When beer is mixed with iron reagent at 85 °C, acetoin in beer is inherently difficult to be determined due to its conversion to diacetyl. During proper fermentation of beer, the concentration of natural acetoin in beer is 100–300 times than that of natural diacetyl in beer. When compared to diacetyl from acetoin, content of natural diacetyl in beer is negligible in determination of acetoin. Therefore, concentration of acetoin could be determined by detecting the concentration of diacetyl (derivative of acetoin) generated by catalytic mechanisms on certain metal oxides, e.g. iron reagent (Cartoni, Coccioli, & Spagnoli, 1997).

Much work has been focused on enhancing detection of diacetyl using dedicated instrumentation with programmed multiple-step operation over the past half century (Casella & Contursi, 2003), and much effort has been devoted to identifying modified approach that may exhibit accurate determination of diacetyl (Guido et al., 2004). Because current popular method contributes to larger relative standard deviations, so it is essential to build a new method to resolve data deviations. In order to obtain an accurate quantification of acetoin in beer, it is necessary to take into consideration any deficiency that occurs during the extraction procedures. The determination of diacetyl in beer is mainly done by gas chromatographic methods (Kondyli, Massouras, Katsiari, & Voutsinas, 2003). Gas chromatography is considered as the most common method for determination and quantitative analysis of acetoin in regular beer. Although several examples of applications are reported (Yasui & Yoda, 1997), no specific accurate methods have been built. Studies display that HS-GC method is plagued by such problem as poor repeatability (Glória & Izquierdo-Pulido, 1999). A sensitive and precise method, based on electron capture chromatography, is described to determine the concentration of acetoin in beer in our laboratory. In this article, the concentration of derivative of acetoin (diacetyl) in beer is measured using a HS-GC with a megabore capillary column and an ECD. The gas chromatography method, headspace auto sampler program, and data handling are all controlled by a system computer and software. This method is applicable to wort, in-process, and packaged beer. Concentration of acetoin in beer can be verified and analysed quantitatively by improving HS-GC techniques. The improving method in

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this article is recognised to be highly selective and sensitive in practices according to the data obtained. The development of analytical methodologies has overcome this challenge for determination and quantitative analysis of acetoin in our laboratory, and data have illustrated ability of this method to reduce the drifts of results.

2. Materials and methods

2.1. Reagents

$FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ were analytical grade and provided by Shanghai Chemical Reagent Company (China). Preparative procedure of iron reagents was performed as follows: 20 mL 14% H_2SO_4 was added into 500 mL purified water, and then 40 g $FeCl_3 \cdot 6H_2O$ and 50 g $FeSO_4 \cdot 7H_2O$ were also dissolved in the purified water. Subsequently, the above solution was diluted to a final volume of 1 L and filtered through rapid velocity filter paper.

2.2. Sample preparation and method of extraction

2.2.1. Sample preparation

The preparation of beer samples were based on the method of Glória and Izquierdo-Pulido (1999) with minor modifications. Briefly, iron reagent was put into a headspace vial (22.3 mL) with a pipette, and then sample was injected into the same vial with a syringe, and subsequently, the vial was tightly sealed immediately with crimp caps and 20-mm white aluminium septa (Supelco). In order to minimise the loss of volatile compounds, the samples were kept at 4 °C when they were not analysed.

2.2.2. Method of extraction

After the sample was placed in a headspace vial containing iron reagent, it was sealed and heated. The iron reagent catalysed acetoin to diacetyl which was evaporated into the vial headspace. The volatile mixture was injected onto a megabore capillary column which separated diacetyl from other compounds after incubation at 85 °C, and the detection of diacetyl was identified with an ECD. Concentration of diacetyl was determined by comparing peak area of diacetyl with a standard curve. The column was agitated at 110 °C and beer samples were incubated during adsorption. The column was exposed to the headspace of the sample for 0.08 min, and nitrogen was used as the carrier gas and kept at a constant flow-rate of 60 mL min^{-1}. The signals of ECD were stored and integrated using computer software (Navigator, Perkin Elmer Corp., USA). Peak identification of diacetyl was based on the retention time of the individual reference standards. Peak integration was performed on a personal computer using PE navigator software. Standard solutions of acetoin were prepared with distilled deionised water.

2.3. GC apparatus

2.3.1. Capillary column

The Capillary column was purchased from Agilent Corporation in German. Chromatographic conditions: Chromatographic separations were acquired at 50 °C using a polyethylene glycol phase capillary column (60 m × 0.53 mm ID × 0.25 μm df) with a 0.25 mm ID × 0.5 m guard column connected w/union (Agilent Co., St Louis, USA). Carrier gas (N_2) was purchased from local suppliers, and its purity was >99.999%.

2.3.2. Chromatography

The chromatographic system was consisted of a headspace analyser (HS40, PE Corp., USA) and gas chromatograph (Clarus500, PE Corp., USA). The concentration of diacetyl was detected by an ECD with a single Ni^{63} radioactive source.

2.4. Statistical analysis

All the data were analysed and submitted to one-way analysis of variance. Differences were considered to be significant at a level of $p < 0.05$ according to SPSS13.0.

3. Results and discussion

3.1. The determination of equilibrium time

The determination of equilibrium time could be carried out under identical conditions by preparing a number of vials with the same beer, incubating them for different time periods. In order to determine equilibrium time of diacetyl, three batches of solution were prepared, and different incubating time periods (ranging from 10 min to 60 min in steps of 5 min) were performed. The apparently optimal equilibrium times were demonstrated in Fig. 1. The appropriate equilibrium time for the mixture of 4.5 mL iron reagent and 0.5 mL sample (batch B) beer was 39 min, and those of the other two solutions were 37 min and 42 min (batch A and C), respectively. Different volumetric iron reagents and beers had various equilibrium times during the incubating process, indicating no uniform equilibrium time in the detection of acetoin in beer by HS-GC.

It was useful for the reduction of equilibrium time to utilise a shaker; equilibrium time was assessed for the mixture of 4.5 mL iron reagent and 0.5 mL beer under identical conditions by detecting diacetyl (Fig. 2). The equilibrium time was 32 min when using a shaker; while no shaker, the equilibrium time of the solution was more than 39 min. The equilibrium time with no shaker was seven more min than that with a shaker. As compared to no shaker, equilibrium time with shaking during the detection of diacetyl was reduced significantly. When a lot of beer sample were analysed, it could save plenty of time to use a shaker, and a better way to decrease equilibrium time was continuous stirring during the incubating process (Liu, Zeng, & Xiong, 2005). In the case of static HS-GC, a shaker was an efficient facility in decreasing equilibrium time. The use of shaker as a tool to curtail incubating time had

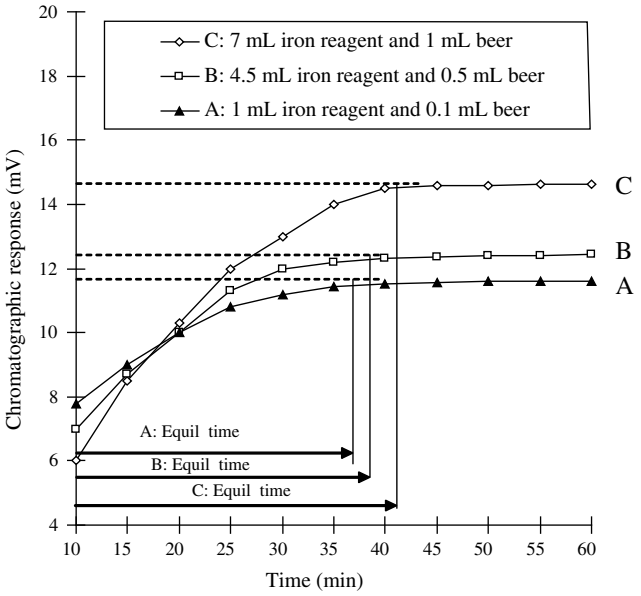

Fig. 1. Determination of equilibrium time of diacetyl (derivative of acetoin) in beer under the condition of different volumetric solutions. Incubating temperature, 85 °C; headspace vials volume, 22.3 mL.

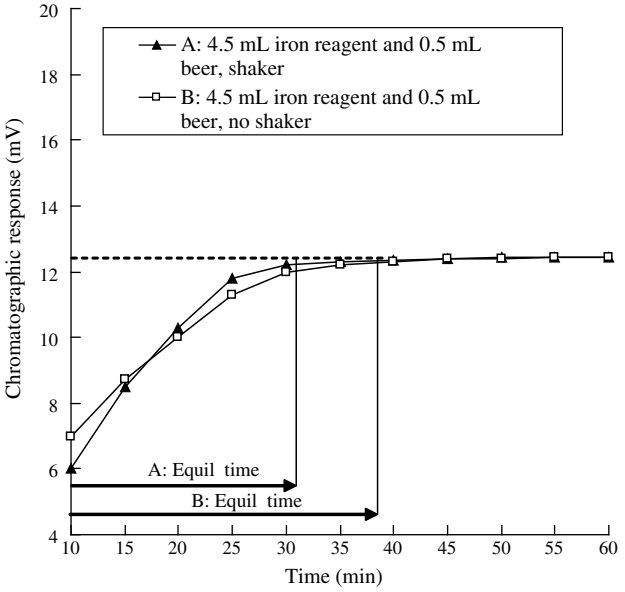

Fig. 2. Evaluations of equilibrium time of diacetyl (derivative of acetoin) in beer under various conditions. A, 4.5 mL iron reagent and 0.5 mL sample with a shaker; B, 4.5 mL iron reagent and 0.5 mL sample with no shaker.

many advantages: it could not only be used as a mixing instrument but also promote whole catalysing reaction (Hill & Smith, 2000).

Inadequate equilibrium time could make the sample unstable between gas phase and liquid phase, and therefore, result in great data variation (Vanhoenacker, De Keukeleire, & Sandra, 2004). The longer equilibrium time help realise the total balance between liquid phase and gas phase, and the scarcity of equilibrium time was responsible for inaccurate detection values of concentration of diacetyl in beer. The time needed for equilibrium depended on the diffusion of volatile sample components (Su, Chang, & Lin, 2004). The analytical result was not changed at equilibrium time or longer time. However, long incubating time should be avoided, because some volatile components were sensitive to prolonged heating (Bvochora, Danner, Miyafuji, Braun, & Zvauya, 2005). It was very necessary to determine the equilibrium time of diacetyl in beer in order to monitor quality of beer (Salemi, Lacorte, Bagheri, & Barceló, 2006).

3.2. Evaluation of calibration: Comparison of 3 levels and 6 levels

The standard addition method was a universal procedure in HS-GC analysis and had been recommended since the early days (Erbe & Brückner, 2000). Standard addition was usually faster to get results than other approaches (Kobayashi, Kusaka, Takahashi, & Sato, 2005). To determine the concentration of diacetyl, we prepared for several samples which contain same volumetric iron reagent and known different concentration standard solutions, then these standard samples were analysed. Multiple level calibration graphs were obtained by plotting peak-area versus known acetoin amount (Coelho, Parrilla, Cervera, Pastor, & de la Guardia, 2003). In order to investigate the efficacy of various level calibrations, solutions of three level of acetoin (1,3 and 6 $\mu g\,mL^{-1}$), as well as 6 levels (0.5,1,1.5,3,6 and 12 $\mu g\,mL^{-1}$), were prepared and calibrated by standard addition calibration, and 2 replications of each level were utilised. Linear regression equations showed that the slope of 3 levels was instable, its change of linear calibration curve exceeded 5%, and it apparently caused bigger deviation (data not shown). However, correlation coefficient of three level calibration was higher than 0.999. The regression equations of 6 levels proved to be more stable than three concentration levels, and slopes of 2 replications of each level were almost identical, even if its correlation coefficient (between 0.99 and 0.999) was lower than that of 3 levels. 6 standard addition samples could avoid deviation as possible as it could when compared to 3 level calibrations. In comparison with 6 level calibrations, 3 levels resulted in inaccurate data of detection. Considering occasional factors during the detection of acetoin, 6 standard level calibration was optimal for the determination of acetoin in beer. It was necessary to add concentration levels so as to pursue data accuracy and concrete process supervision (Pasteris & Strasser de Saad, 2005).

3.3. Pressure configuration

Extraction techniques of diacetyl (derivative of acetoin) in beer required to set up pressure parameters of headspace and gas chromatograph for HS-GC (Lasekan, Buettner, & Christlbauer, 2007). We built up two batches of pressure parameters: HS (30 psi)/GC (25 psi) and HS (30 psi)/GC (22 psi), and comparisons of values were shown in Figs. 3 and 4. Fig. 3 was the single value control chart which indicated process control capacity during detection of diacetyl. Batch A was the variation of concentration of diacetyl under the condition of pressure of HS (30 psi)/GC (25 psi), and batch B was the variation of data under the condition of pressure of HS (30 psi)/GC (22 psi). The average of concentration of batch A was higher than that of batch B, and it was attributable to the drift of data aroused from lower precision. In contrast to batch A, measuring precision of batch B was significantly improved to $\leqslant$2% R.S.D. The data obtained displayed that detection had a higher process control capacity under the condition of HS (30 psi)/GC (22 psi) in comparison with HS (30 psi)/GC (25 psi). Fig. 4 displayed the range control chart of diacetyl. The threshold of concentration of batch A was higher than that of batch B, indicating process control capacity in batch A lower than batch B. The comparison of range between batch A and B illustrated that it was more potent to overcome the problems of detection precision under the condition of HS (30 psi)/GC (22 psi). Batch A had higher deviation (5% R.S.D), which induced inaccurate conclusion and significant variations of data ($p < 0.05$) in the verification of beer quality. With respect to reproducibility and detection limit, performance under the condition of HS (30 psi)/GC (22 psi) was satisfactory when

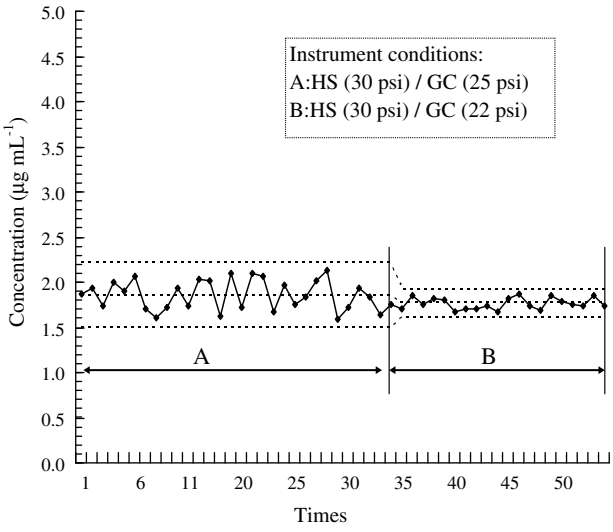

Fig. 3. Single value control chart of diacetyl (derivative of acetoin) versus times. A, HS (30 psi)/GC (25 psi); B, HS (30 psi)/GC (22 psi). HS (30 psi) represents that the pressure of the injector in HS is 30 psi; GC (25 psi) represents that the pressure of the capillary column inlet in GC is 25 psi; GC (22 psi) represents that the pressure of the capillary column inlet in GC is 22 psi.

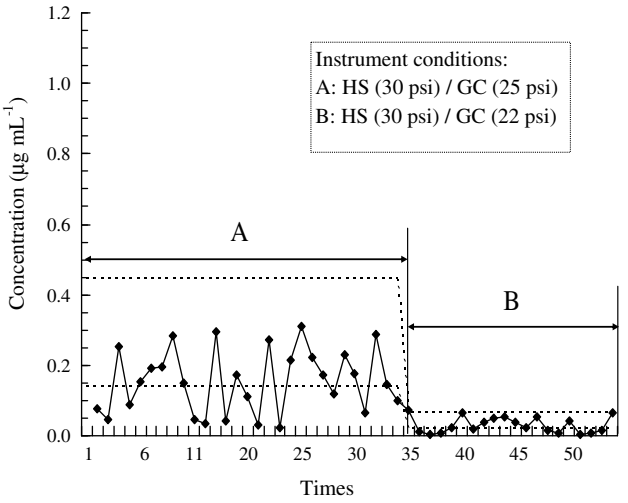

Fig. 4. Range control chart of diacetyl (derivative of acetoin) versus times. A, HS (30 psi)/GC (25 psi); B, HS (30 psi)/GC (22 psi); HS (30 psi) represents that the pressure of the injector in HS is 30 psi; GC (25 psi) represents that the pressure of the capillary column inlet in GC is 25 psi; GC (22 psi) represents that the pressure of the capillary column inlet in GC is 22 psi.

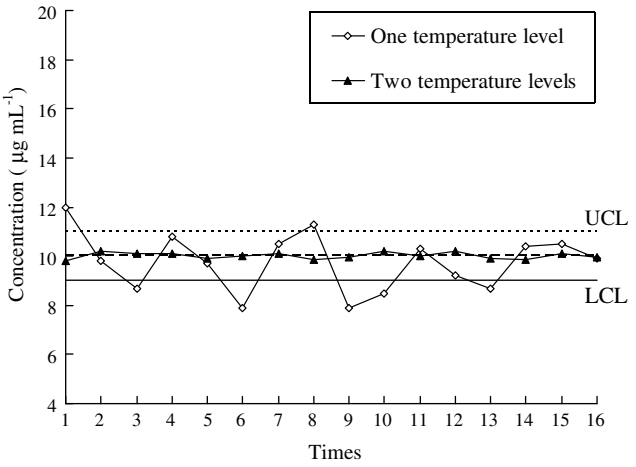

Fig. 5. The detection values of diacetyl (derivative of acetoin) under the conditions of various temperature levels. UCL, the upper control limit of diacetyl; LCL, the lower control limit of diacetyl.

compared to HS (30 psi)/GC (25 psi). Higher process control capacity of batch B demonstrated that parameter configuration of HS (30 psi)/GC (22 psi) superior to that of HS (30 psi)/GC (25 psi). To optimise the conditions of accurate analysis, the pressure HS (30 psi)/GC (22 psi) should be adopted.

Introduction volume, transferred into the capillary column for separation and analysis from headspace of vials, depended on the carrier gas pressure of the column inlet and the time of being transferred (Bvochora et al., 2005), and various pressures resulted in different introduction volume (Cartoni et al., 1997; Chyau, Ko, Chang, & Mau, 2003). The introduction volume was displayed in Table 1. Volatile diacetyl in headspace was evaporated into column after the mixture of beer and iron reagent was incubated for 40 min at 85 °C. Because of different pressure at headspace and gas chromatograph, volatile volumetric diacetyl was finally transferred into the cell containing an ECD and detected (Gorinstein et al., 1999; Kondyli, Katsiari, Masouras, & Voutsinas, 2002). The results displayed that pressure variation did significantly affect the introduction volume ($p < 0.05$).

3.4. Temperature level configuration

In order to optimise the choice of operational applied potential, a study about configuration of temperature levels was considered. It was essential to prepare a series of identical beer samples to obtain accurate parameter. All samples were composed of 4.5 mL iron reagent and 0.5 mL sample beer, and they were incubated at 85 °C for 32 min with the stirring of a shaker. The accuracy of data was observed by detecting the concentration of diacetyl in beer versus injecting times (Fig. 5). The maximum of concentration was 12.1 μg mL^{-1} under the condition of one level, and the minimum was 7.9 μg mL^{-1}, and therefore, its range was 4.2 μg mL^{-1}. As compared to one level process, two levels exhibited a more stable signal which range was 0.4 μg mL^{-1} (⩽1.5% R.S.D) in beer, and there was a diminutive range for two levels in comparison with one level. A lot of detecting values under the condition of one level exceeded the required range and did not satisfy detection requirements. Temperature configuration of two levels provided optimal experimental conditions. In comparison with one level, temperature configuration of two levels was generally reliable and suitable for the determination of concentration of diacetyl. When level configurations were multiple levels, good performance could be obtained (Vanhoenacker et al., 2004).

It was possible for injector, capillary column and detector to be polluted when lots of beer samples were analysed (Glória & Izquierdo-Pulido, 1999; Lanciotti, Patrignani, Iucci, Saracino, & Guerzoni, 2007). Frequent instrument maintenance should be required in view of the potential problems resulted from repeated injections, such as contamination of the liner by non-volatile components (Gorinstein et al., 1999). Because of complex composition of beer, tedious cleanup of the extract was generally required before GC analysis (Pasteris & Strasser de Saad, 2005). Direct analysis of diacetyl had been used to overcome the interference of the chemical background from a crude beer extract with two temperature levels. This instrument approach was applicable for long sequence of analysis, and accurate analysis results could be obtained, because it cleared up the pollutants caused by repeated injections of the complex extract.

Table 1
The volume of volatile sample transferred into the capillary column

Parameters	HS (30 psi)/GC (25 psi)	HS (30 psi)/GC (22 psi)
Column outlet (atmospheric) pressure: Pa	101.3 kPa	101.3 kPa
Column head pressure: ΔP	172.41 kPa	151.72 kPa
Column inlet pressure: Pi = ΔP + Pa	274.71 kPa	253.02 kPa
Measured flow rate at column outlet: Fa	27.8 mL min^{-1}	18.5 mL min^{-1}
Vial temperature (85 °C): Tv	358.16 K	358.16 K
Partial vapour pressure of water at ambient temperature: Pw	2.637 kPa	2.637 kPa
Total flow rate: Fc.o = Fa* (Tv/Ta)* (Pa-Pw)* Pa	28.8 mL min^{-1}	19.6 mL min^{-1}
Partial flow rate: Fi = (Pa/Pi)* Fc.o	8.36 mL min^{-1}	7.52 mL min^{-1}
Volume of sample: Vgas = Fi* t	0.83 mL	0.61 mL

1 psi = 6.895 kPa.

4. Conclusion

In this article, HS-GC was used to determine the concentration of diacetyl (derivative of acetoin) for the determination and quantitative analysis of acetoin in beer. Through investigation, the proposed analytical method appeared to be appropriate for the routine applications regarding to determination of acetoin present in beer, and analysis of diacetyl by HS-GC with an ECD had been found feasible. The good sensitivity and recoveries confirmed the potential interest of this analytical strategy in real analytical contexts.

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