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Application News

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Gas Chromatography

Analysis of Diacetyl and 2,3-pentanedione in Beer Using Nexis™ GC-2030

Among the off-flavors in beer, there are diacetyl and 2,3-pentanedione (collectively known as vicinal diketone: VDK). More than a certain level of VDK, which is generated during fermentation, gives beer its "butterscotch" aroma. The concentration of VDK needs to be controlled to prevent it from affecting the flavor of the beer. This report presents the quantitative results for VDK and changes in VDK level before and after main fermentation in two commercially available beer samples.

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Reagent Preparation/Quantitative Method

The standard solutions were prepared at concentrations of 25 ppb, 50 ppb and 100 ppb (v/v) by diluting diacetyl and 2,3-pentanedione with water. For preparation of the internal standard solution, 2,3-hexadione aqueous solution was added to each standard solution at 50 ppb (v/v). After pouring 5 mL of each sample into a headspace vial and sealing the vial, headspace analysis was performed and calibration curves were prepared. For preparation of the sample for analysis, the internal standard solution was added to non-degassed beer samples, as described above. After 5 mL of each sample was poured into a headspace vial that was then sealed and heated at 60 °C for 90 minutes and subsequently cooled for determination of total amount of VDK, including precursors*1, these vials were placed in the sample tray on HS-20 to perform headspace analysis and quantification using the prepared calibration curves.

*1 Beer samples were heated in an oven as a pretreatment prior to analysis because the precursors are converted into VDK by heating.

Instrument Configuration and Analytical Conditions

The instrument configuration and analytical conditions are shown in Table 1.

Table 1 Instrument Configuration and Analytical Conditions

HS-20		
Mode	:	Loop
Oven Temperature	:	40 °C
Sample Line Temperature	:	90 °C
Transfer Line Temperature	:	95 °C
Vial Pressure	:	150 kPa
Vial Heat-retention Time	:	40 min
Vial Pressurization Time	:	1 min
Vial Pressurization Equilibrating Time	:	0.1 min
Loading Time	:	0.5 min
Loading Pressurization Time	:	0.1 min
Injection Time	:	0.5 min
Needle Flush Time	:	5 min
Nexis GC-2030 / ECD-2010 Exceed		
Injection Mode	:	Split
Split Ratio	:	1:20
Carrier Gas	:	He
Carrier Gas Control	:	Constant Linear Velocity (40 cm/sec)
Column	:	SH-Rtx™-624 (60 m × 0.32 mm I.D., 1.80 µm)
Column Temp	:	50 °C - 5 °C/min – 120 °C (6 min)
Detector Temp	:	130°C
ECD current	:	2.5 nA
Detector Gas	:	N_2 15 mL/min

Chromatogram and Calibration Curves of Standard Samples

The chromatogram and calibration curves of standard samples are shown in Fig. 1 and Fig. 2, respectively.

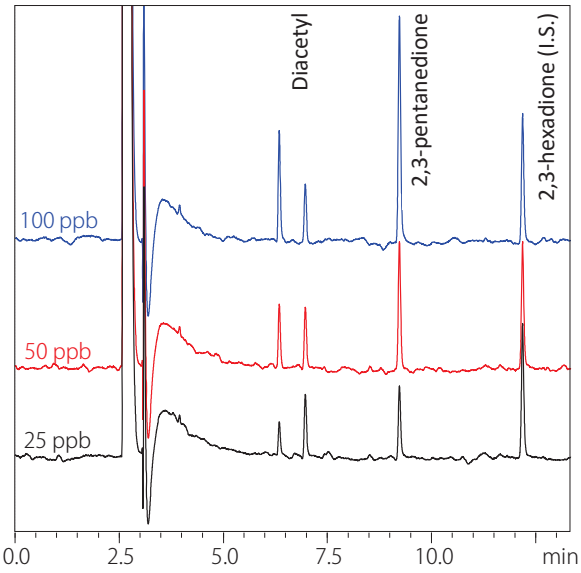

Fig. 1 Chromatogram of Standard Samples

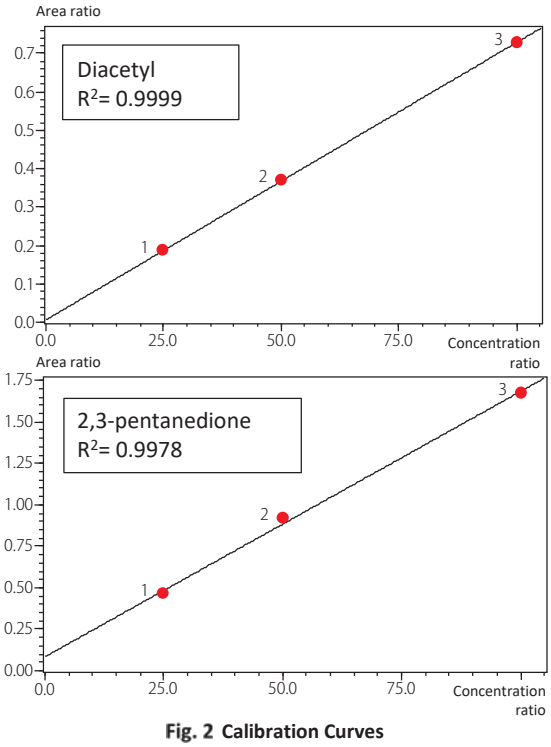

Fig. 2 Calibration Curves

Quantitative Results for VDK in Two Commercially Available Beer Samples

The quantitative results for VDK in two commercially available beer samples are shown in Table 2. The representative chromatogram is shown in Fig. 3.

Table 2 Quantitative Results for VDK in Two Brands of Beer Samples

Beer	Diacetyl	2,3-pentanedione
A	18.2 ppb	1.8 ppb
B	39.7 ppb	7.1 ppb

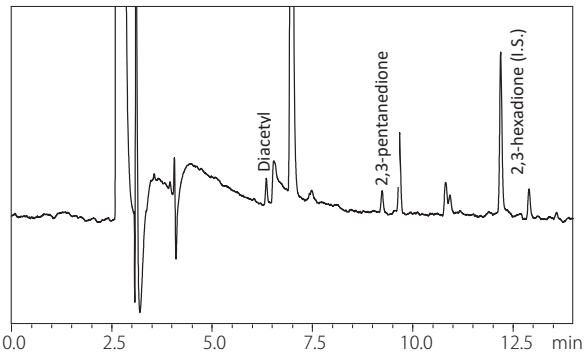

Fig. 3 Chromatogram of Beer Samples

Changes in VDK Level After Main Fermentation

After main fermentation, the diacetyl rest process (a process in which yeasts are allowed to consume VDK) is completed, followed by the maturation process. The quantitative results and chromatogram of VDK in beer samples during the diacetyl rest process are shown in Table 3 and Fig. 4, respectively.

The VDK peak decreases as the duration of main fermentation increases. This demonstrated the consumption of diacetyl by yeasts and successful diacetyl rest.

In addition, the quantitative results for VDK in beer samples before bottling after the completion of the maturation process are also shown.

Table 3 Quantitative Results for VDK in Beer Samples After Main Fermentation

Duration after the completion of main fermentation	Diacetyl	2,3-pentanedione
Day 4	208.9 ppb	32.1 ppb
Day 5	130.6 ppb	18.6 ppb
Day 6	101.7 ppb	12.0 ppb
Before bottling	39.7 ppb	7.1 ppb

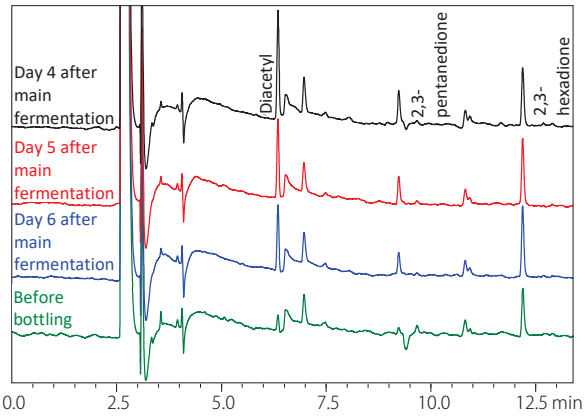

Fig. 4 Chromatogram of Beer Samples After Main Fermentation and Before Bottling

Conclusion

VDK that may affect flavor and aroma of beer has primarily been determined by sensory evaluation.

Meanwhile, headspace analysis using gas chromatography enables easy quantitative analysis of even low concentrations of VDK without special pretreatment such as degassing or concentration.

Fig. 5 Appearance of Nexis™ GC-2030 + HS-20

<Reference>
Narihiro Suzuki: Brewer Produces World's Most Delicious Beer with Wild Yeast; Shinchosha (2019)

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