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Application News

GC-MS GCMS-TQ™8040 NX

Integrated Analysis of Aromatic Components and Metabolites in Beer Samples Using GC-MS Smart Databases

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User Benefits

- The aromatic components and metabolites in samples can be analyzed comprehensively using each Smart Database.
- Integrated analysis can be performed using the SIMCA® 17 multivariate analysis program (INFOCOM CORPORATION), which is capable of processing multiple datasets from the same samples obtained by different analyses.

■ Introduction

Evaluating the flavor of foods is essential for quality control and product development, and can be carried out in a variety of ways. One example is the comprehensive analysis of aromas and metabolites in food products. The results obtained can be used in combination as indicators of flavor and functionality.

Shimadzu provides various preconfigured databases to assist users in smoothly initiating GC-MS(/MS) analysis. In this article, aromatic components and metabolites in commercially available beers and test beers under development were comprehensively analyzed using Smart Aroma Database and Smart Metabolites Database. The characteristics of each of the samples were visualized by processing the data using multivariate analysis. Furthermore, in order to investigate the correlation between aromatic components and metabolites, an integrated analysis of these two datasets was also performed.

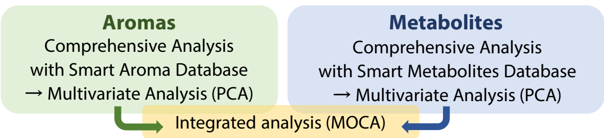

Fig. 1 Analysis Sequence in this Article

■ Smart Aroma Database

Smart Aroma Database enables the comprehensive detection of trace amounts of aromas and the identification of each component's contribution to the fragrance. By obtaining SCAN chromatograms using a method created from this database, approximately 500 principal aromas can be identified. The odor characteristics and semi-quantitative values in the database can be used to identify the main components contributing to the fragrance.

Comprehensive analysis is easily initiated following the analysis of an n-alkane standard sample for the purpose of retention time modification. A variety of pretreatment tools are supported including HS-20 NX, AOC-30i, AOC-6000 Plus (HS, SPME, SPME Arrow), TD-30, OPTIC-4, and OP275 Pro. The database analysis conditions used in this article are shown in Table 1.

■ Smart Metabolites Database

Smart Metabolites Database is specialized for metabolites. As with Smart Aroma Database, the proper measurement conditions are easily configured following the analysis of an n-alkane standard sample. The database enables the comprehensive analysis of approximately 600 pre-registered metabolites, in foods and biological samples for example.

The database analysis conditions used in this article are shown in Table 2. Most of the metabolites are not volatile, so they need to be methoximated and trimethylsilylated before they can be analyzed with a GC-MS. Refer to Application News M280 (LAAN-A-MS063A) for the derivatization procedures in detail.

Table 1 Analysis Conditions with Smart Aroma Database

Model		: GCMS-QP™2020 NX
Autosampler		: HS-20 NX
Column		: InertCap Pure-wax (30 m × 0.25 mm I.D. 0.25 µm)
[HS]	Mode	: Trap (Tenax®TA 60/80 mesh)
	Oven Temperature	: 60 °C (Commercially Available Beers) 40 °C (Test Beers under Development)
	Multi Injection Times	: 5
	Sample Line Temperature	: 100 °C
	Transfer Line Temperature	: 100 °C
	Trap Cooling Temperature	: -10 °C
	Trap Heating Temperature	: 280 °C
	Trap Waiting Temperature	: 25 °C
	Vial Pressure	: 80 kPa
	Dry Purge Pressure	: 60 kPa
	Vial Heat-retention Time	: 30 min
	Vial Pressurization Time	: 1 min
	Vial Pressurization Equilibrating Time	: 0.1 min
	Loading Time	: 1 min
	Loading Pressurization Time	: 0.1 min
	Dry Purge Time	: 10 min
	Injection Time	: 3 min
	Needle Flush Time	: 5 min
[GC]	Injection Mode	: Split (1:5)
	Carrier Gas	: He, Constant Pressure (83.5 kPa)
	Column Oven Temperature Program	: 50 °C (5 min) – 10 °C/min – 250 °C (10 min)
[MS]	Ion Source Temp.	: 200 °C
	Interface Temp.	: 250 °C
	Acquisition Mode	: Scan/SIM
	Event Time	: 0.3 sec (Scan), 0.2 sec (SIM)
	m/z range	: 35 – 400 (Scan)

Table 2 Analysis Conditions with Smart Metabolites Database

Model		: GCMS-TQ™8040 NX
Autoinjector		: AOC-20i+s
Column		: DB-5MS (30m×0.25mm I.D., 1.0µm)
[GC]	Injection Temp.	: 280 °C
	Injection Mode	: Spitless
	Sampling Time	: 1 min
	Carrier Gas	: He, Constant Liner Velocity (39 cm/s)
	Purge Flow	: 5.0 mL/min
	Column Oven Temperature Program	: 100 °C (4 min) – 10 °C/min – 320 °C (11 min)
	Injection Volume	: 1 µL
[MS]	Interface Temp.	: 200 °C
	Ion Source Temp.	: 280 °C
	Acquisition Mode	: MRM
	Loop Time	: 0.3 sec

■ Commercially Available Beers [1] Analysis of Aromatic Components

As a quality control indicator, the aromatic components in 9 commercially available beers were previously analyzed in Application News 01-00137. A total of 143 components were identified via a comprehensive analysis with Smart Aroma Database. The differences between the beers were visualized with Principal Component Analysis (PCA) using SIMCA17 (INFOCOM CORPORATION), a multivariate analysis program (Fig. 2).

Table 3 Commercially Available Beers Analyzed in this Article

Beer Samples		
• Ale1	• White Ale	• Lager1
• Ale2	• IPA	• Lager2
• Ale3	• Barrel Aged	• Lager3

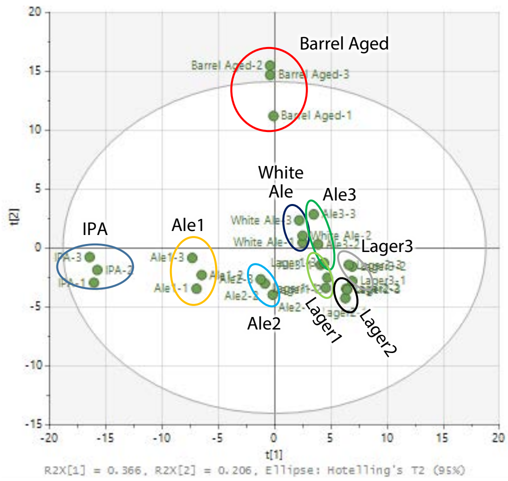

Fig. 2 PCA Score Plot for Data from the Analysis of Aromatic Components in 9 Commercially Available Beers

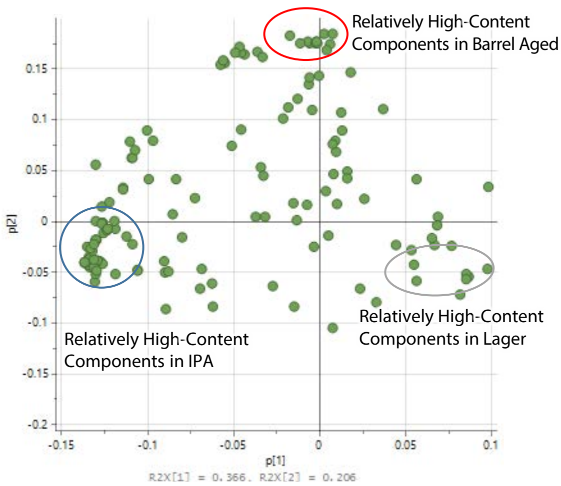

Fig. 3 PCA Loading Plot for Data from the Analysis of Aromatic Components in 9 Commercially Available Beers

Table 4 Examples of Relatively High-Content Aromatic Components in Each Sample

Beer Sample	Relatively High-Content Aromatic Components
Lager3	2-Methylbutyl acetate, Isoamyl acetate, 2-Phenylethyl acetate
IPA	Linalool, Geraniol, Nerol, Ethyl heptanoate, 2-Nonanol, Terpinen-4-ol
Barrel Aged	Ethyl lactate, Acetic acid, Benzaldehyde, Diethyl succinate

■ Commercially Available Beers [2] Analysis of Metabolites

The same samples from [1] were methoximated and trimethylsilylated, after which a comprehensive analysis was performed using the conditions in Smart Metabolites Database. A total of 375 metabolites were identified, and as with the analysis of aromatic components, the differences between the 9 commercially available beers were visualized using PCA (Fig. 4).

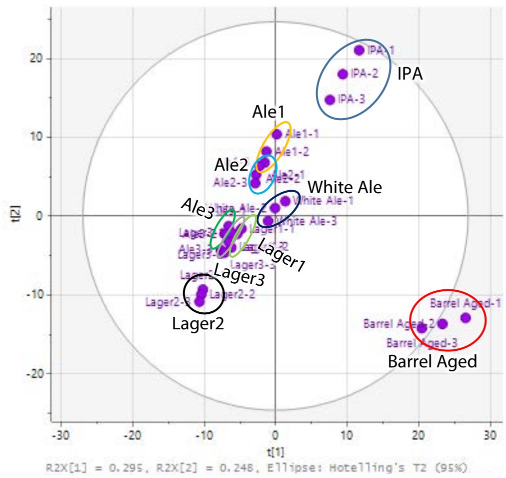

Fig. 4 PCA Score Plot for Data from the Analysis of Metabolites in 9 Commercially Available Beers

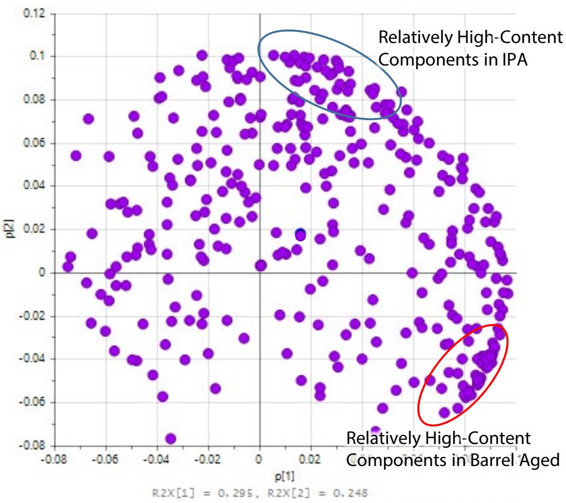

Fig. 5 PCA Loading Plot for Data from the Analysis of Metabolites in 9 Commercially Available Beers

Table 5 Example of Relatively High-Content Metabolites in Each Sample

Beer Sample	Relatively High-Content Metabolites
IPA	Xylitol, Inositol, Histidine, Glucaric acid, Asparagine, Aconitic acid
Barrel Aged	Lactic acid, Uracil, Arabitol, Mannitol, Tartaric acid, Leucine

■ Test Beers under Development [1] Analysis of Aromatic Components

For the purpose of selecting primary new yeasts in the development of new beers brewed using wild yeast, newly discovered yeast (New Yeast) and previous ones (Yeast1, Yeast2) were compared. A comprehensive analysis of the aromatic components in the beer samples was performed using Smart Aroma Database, and 140 components were identified. Using PCA, the difference between the new yeast and the conventional yeasts was confirmed (Fig. 6).

Fig. 6 PCA Score Plot for Data from the Analysis of Aromatic Components in Test Beers

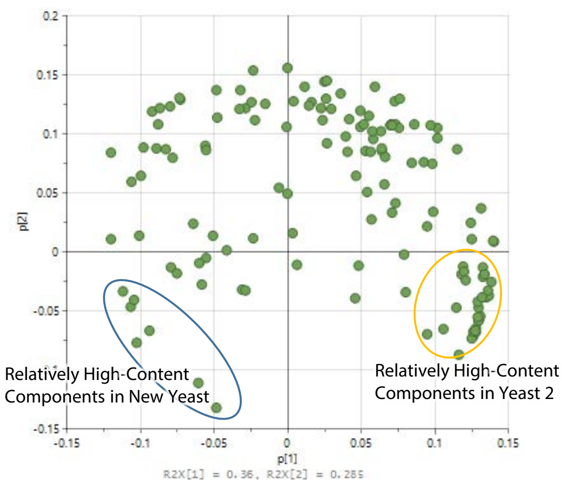

Fig. 7 PCA Loading Plot for Data from the Analysis of Aromatic Components in Test Beers

Table 6 Example of Relatively High-Content Aromatic Components in Each Sample

Beer Sample	Relatively High-Content Aromatic Components
New Yeast	Linalyl butyrate, alpha-Humulene, Isobutyl acetate
Yeast2	Ethyl acetate, Methyl octanoate, Neral, Methyl geranate, d-Carvone, 4-Methoxystyrene

■ Test Beers under Development [2] Analysis of Metabolites

The same samples as [1] were methoximated and trimethylsilylated, after which a comprehensive analysis was performed using the conditions in Smart Metabolites Database. A total of 361 metabolites were identified. The difference between the metabolites in beers brewed from wild yeasts and the other beers was visualized using PCA (Fig. 8).

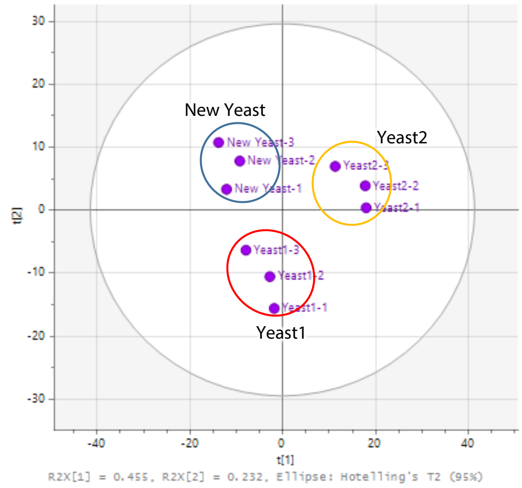

Fig. 8 PCA Score Plot for Data from the Analysis of Metabolites in Test Beers

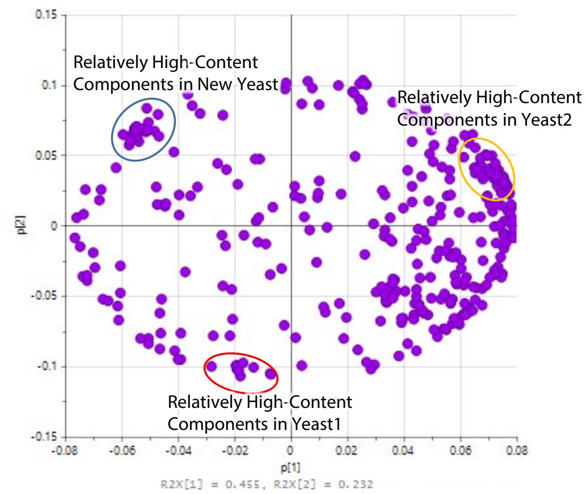

Fig. 9 PCA Loading Plot for Data from the Analysis of Metabolites in Test Beers

Table 7 Example of Relatively High-Content Metabolites in Each Sample

Beer Sample	Relatively High-Content Metabolites
New Yeast	Maltose, Putrescine, Glycine, 4-Hydroxyproline, Glutamine, Glycerol
Yeast1	Sucrose, Isomaltose, Glutaric acid
Yeast2	4-Aminobutyric acid, Pyruvic acid, Mannose, Xylitol, 3-Aminopropanoic acid, Lysine

■ Integrated Analysis Using MOCA

MOCA, which stands for Multiblock Orthogonal Component Analysis, is a new method for analyzing multiple sets of data (multiblock data), obtained from the same sample using different methods. It is useful for investigating the positive and negative correlations between multiblock datasets.

An integrated analysis was performed of the 2 datasets obtained through comprehensive analysis using each of the Smart Databases, and the correlation between the aromatic components and metabolites was evaluated.

• Commercially Available Beers

In Figs. 2 and 4, the characteristics of 9 different beers were visualized separately using a PCA of the data from the aroma and metabolite analyses. MOCA was then used to clarify the correlation between the two factors. The circle indicating each sample was smaller than the PCA score plot, which means that there was only a small difference between the scores for each data block (for the aromatic components and metabolites). This implies a high correlation between the aromatic components and metabolites. For example, the aromatic component Linalool and the metabolite Inositol were both present at high concentrations in IPA, which was clearly different than in the other samples.

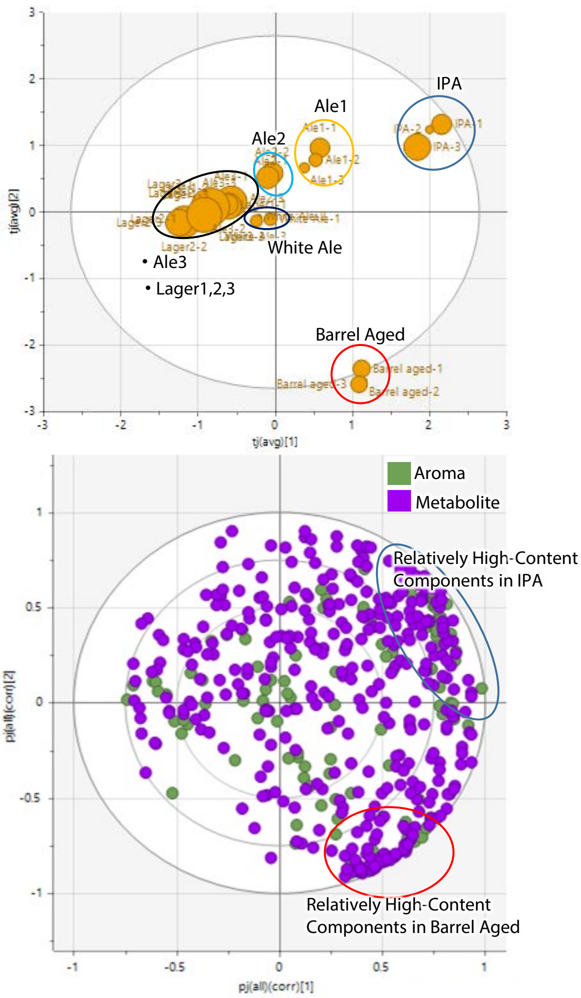

Fig. 10 MOCA Plot for Data from the Analysis of Aromatic Components and Metabolites in 9 Commercially Available Beers

• Test Beers under Development

The circles indicating each sample of the beers were also small, implying a high correlation between aromatic components and metabolites. For example, in the New Yeast data, the aromatic component alpha-Humulene and the metabolite Glycine were prevalent and more correlated than in other samples.

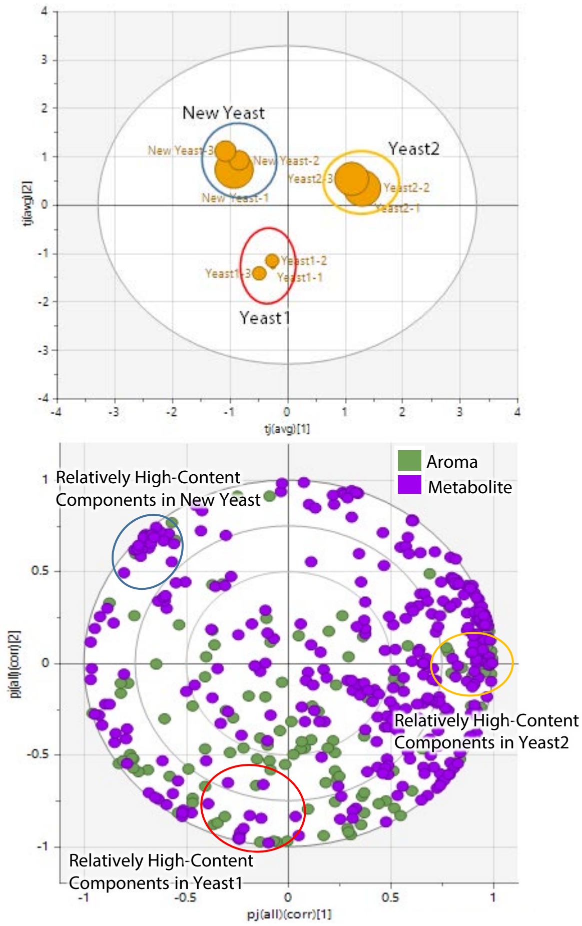

Fig. 11 MOCA Plot for Data from the Analysis of Aromatic Components and Metabolites in Test Beers

■ Conclusion

This article has presented a comprehensive analysis of the aromatic components and metabolites in beer samples. Despite the number of target components, the analysis was performed efficiently using the Smart Databases. Multivariate analysis of the datasets obtained from the comprehensive analysis provided a method of visualizing the differences between the samples. Furthermore, the integrated analysis using MOCA indicated the correlations between the aromatic components and metabolites. The data acquired could be useful not only for quality control with respect to commercially available beers, but also for the selection of yeasts used for beers under development.

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