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Application News

Gas Chromatograph Mass Spectrometers GCMS-QP2020 NX and GCMS-TQ™8040 NX
High Performance Liquid Chromatograph Mass Spectrometers LCMS-8060NX and LCMS-9050

Aroma and Metabolite Analysis Using GC-MS and LC-MS and Approach to Craft Beer Development

Yuto Nakasuji, Ayako Nomura, Tetsuo Iida, Misato Ishimoto, Yoshihiro Saito, and Yusuke Takemori

User Benefits

- The Smart Aroma Database™ and Smart Metabolites Database™ Ver. 2 can perform comprehensive analysis of aroma and metabolite compounds.
- The Multi-Omics Analysis Package can efficiently analyze GC-MS and LC-MS metabolomic data.
- The LC-QTOF can perform attribute analysis of unidentified compounds.

■ Introduction

Craft beers are beers that are brewed in small batches and enjoyed around the world. Craft breweries use select techniques and ingredients, such as barley and hops, to create beers that are enjoyed for their unique and varied aromas and tastes. Beer flavor analysis is performed using various methods and is an essential process in the development and quality control of beers. In recent years, there has been growing interest in using analytical instruments, rather than conventional human sensory evaluations, to objectively evaluate food flavors and functions by comprehensively analyzing aromas and metabolite compounds.

In this article, beers were analyzed using gas chromatography-mass spectrometer (GC-MS) and liquid chromatography-mass spectrometer (LC-MS). First, aroma compounds were analyzed using GC-MS. Next, complementary analysis of metabolite compounds in common samples was performed using targeted metabolomics with GC-MS and LC-MS. Finally, attribute analysis (non-targeted metabolomics) was performed on unidentified metabolites in the samples, based on the determination of the exact mass using quadrupole time-of-flight LC-MS (LC-QTOF). The compounds that can be detected by GC-MS and by LC-MS differ, so using both techniques makes it possible to complement the analytical data. The resulting data was then used to develop new craft beers.

■ Samples Analyzed

Experimental brewing was performed to create craft beers featuring American Ale and London Ale yeast strains and two types of naturally derived wild yeast (Fig. 1). The same ingredients and brewing conditions were used for each type of beer. The instruments used for aroma and metabolite analysis are shown in Fig. 2. The following four beer samples, each containing a different yeast strain, were used in the analyses:

(1) Wild Yeast 1
(2) Wild Yeast 2
(3) London Ale yeast (commercial yeast)
(4) American Ale yeast (commercial yeast)

(1) (2) (3) (4)

Fig. 1 Yeast Strains and Beer Samples Used in Experimental Brewing

• Aroma analysis

HS-20 NX + GCMS-QP2020 NX
Single-Quadrupole GC-MS

• Metabolite analysis (targeted metabolomics)

GCMS-TQ™8040 NX
Triple-Quadrupole GC-MS

Nexera™ X3 + LCMS-8060NX
Triple-Quadrupole LC-MS

• Metabolite analysis (non-targeted metabolomics)

Nexera™ X3 + LCMS-9050
Quadrupole Time-of-Flight (QTOF) LC-MS

Fig. 2 Instruments Used

■ Aroma Analysis Using the Smart Aroma Database

Aroma is a key factor for determining product value in the development of foods and beverages, and analysis of aroma compounds (aroma analysis) is an essential process in controlling product aromas. Aromas that are perceptible to humans are complexes consisting of numerous diverse aroma compounds. So aroma analysis requires not only the analysis of simple compounds but also the characterization of compounds that significantly contribute to the aroma.

The Smart Aroma Database assists with research into correlations between aroma compounds and smells. Its scan analysis feature and proprietary aroma compound library enable highly accurate screening of around 500 types of aroma compounds. It also supports selected ion monitoring (SIM) and multiple reaction monitoring (MRM) modes, which facilitate detailed and targeted analysis of compounds and known important compounds that are selected from multivariate analysis. The database includes analytical methods compatible with sniffer units, so it can be used to evaluate the correlation between compounds and smells.

The results of aroma analysis obtained using the Smart Aroma Database can be used not only to improve test products but also for the promotion and branding of products (Fig. 3).

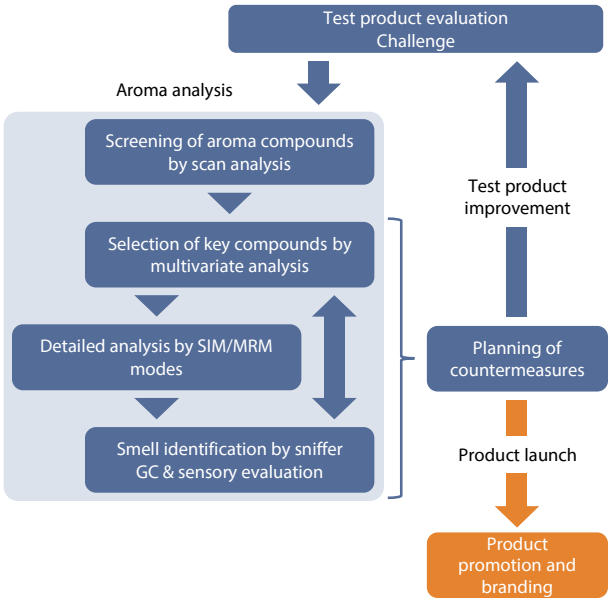


Fig. 3 Workflow Example Using Smart Aroma Database

■ Samples and Analytical Conditions for Aroma Analysis

Aroma analysis was performed in trap mode using a system configuration that linked the GCMS-QP2020 NX single quadrupole GC-MS and the HS-20 NX headspace sampler (Fig. 4).

The samples were four types of beers experimentally brewed using different yeast strains. For each beer, 5 g of the sample and 3 g of NaCl were sealed in 20 mL crimp vials and set on the HS-20 NX for analysis. Each sample was measured three times consecutively. In the trap mode of the HS-20 NX, the headspace gas from the beer sample is concentrated into a trap tube and automatically introduced into the GC (Fig. 5). This feature eliminates the need for complex pretreatment of samples, and it enables highly sensitive analysis of aroma compounds with only the simple preparation of sealing the sample into the vial. The analytical conditions are listed in Table 1.

Fig. 4 HS-20 NX + GCMS-QP2020 NX

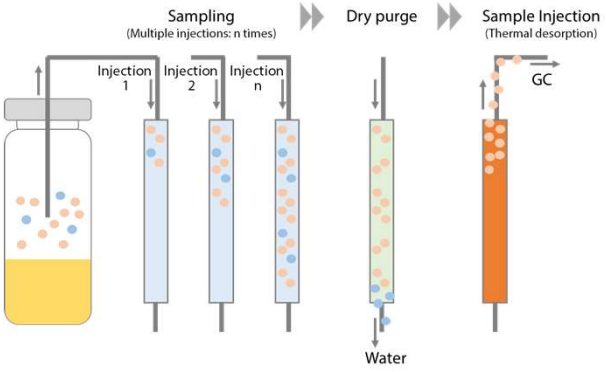


Fig. 5 Headspace Sampling in Trap Mode

Table 1 Instrument Configuration and Analytical Conditions

<GC-MS>	GCMS-QP2020 NX
<Head Space Sampler>	HS-20 NX

<HS>

Mode:	Trap (Tenax TA)
Oven Temp.:	60 °C
Sample Line Temp.:	100 °C
Transfer Line Temp.:	100 °C
Trap Cooling Temp.:	-10 °C
Trap Heating Temp.:	280 °C
Trap Waiting Temp.:	25 °C
Multi Injection:	5
Vial Pressure:	80 kPa
Dry Purge Pressure:	60 kPa
Vial Heating Time:	30 min
Vial Pressurization Time:	1 min
Pressure Equilibrating Time:	0.1 min
Loading Time:	1 min
Load Equilibrating Time:	0.1 min
Dry Purge Time:	10 min
Injection Time:	3 min
Needle Flush Time:	5 min

<GC>

Injection Mode:	Split
Split Ratio:	5
Carrier Gas:	He
Carrier Gas Control:	Const. Pressure (83.5 kPa)
Column:	InertCap Pure-WAX (30 m, 0.25 mm i.d., df = 0.25 µm)
Oven Program:	50 °C (5 min)→10 °C/min→250 °C (10 min)

<MS>

Ion Source Temp.:	200 °C
Interface Temp.:	250 °C
Data Acquisition Mode:	Scan (*m/z* 35 - 400)
Event Time:	0.3 sec

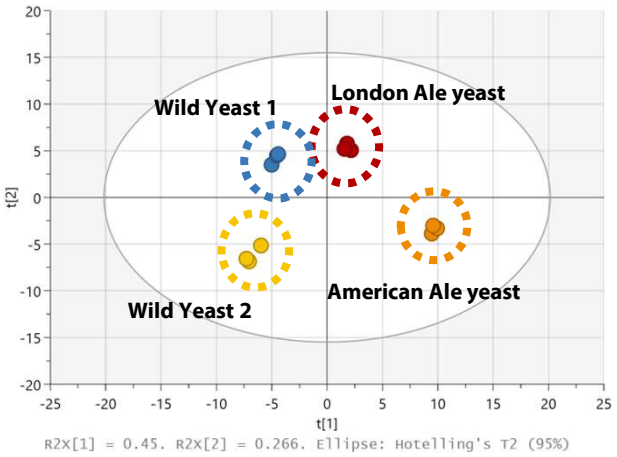
■ Aroma Analysis Using GC-MS

In aroma analysis, four beer samples brewed with different yeasts were analyzed using the Smart Aroma Database. The analysis was performed in scan mode, and 100 compounds were identified from the four beer samples.

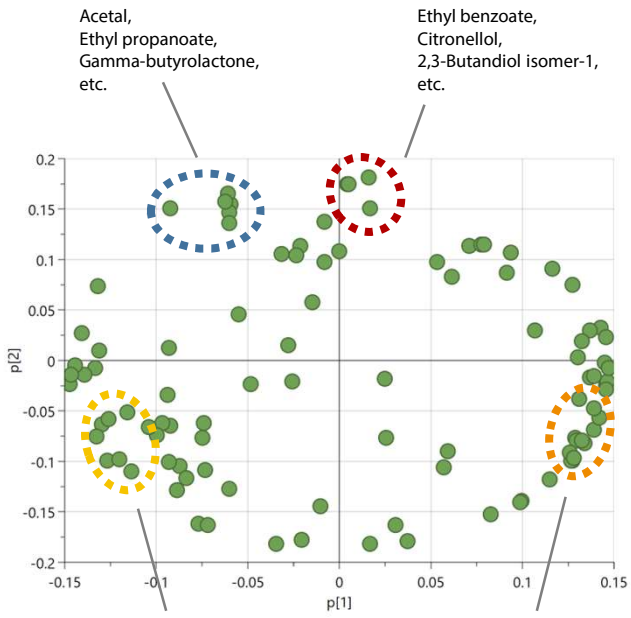
Principal component analysis (PCA) was then performed using the areas of these compounds to visualize the differences between each sample and their respective characteristic compounds. (SIMCA 18 multivariate data analysis software [Infocom Corporation] was used in the PCA.) The results of the PCA are shown in Fig. 6.

On the score plot in Fig. 6-(a), the four samples are separated into four clusters by the first principal component (PC1) and second principal component (PC2) axes. As can be seen, the two commercial yeast beer samples are plotted in the positive direction of PC1, while the two wild yeast beer samples are plotted in the negative direction of PC1. This suggests that there was a significant difference in the aroma compounds of the commercial and wild yeast strains, which explains the different flavor characteristics.

The aroma compounds with relatively high concentrations in each beer sample were confirmed from the loading plot. Fig. 6-(b) shows the characteristic aroma compounds present in each yeast strain.



(a) Score Plot



(b) Loading Plot

Fig. 6 Results of PCA of Detected Aroma Compounds

In this analysis, particularly high concentrations of *p*-vinylguaiacol (4-vinylguaiacol), styrene, and beta-damascenone were detected in the beer sample with Wild Yeast 2. This can be seen in Fig. 7, which compares the mass chromatograms of these compounds in each beer sample. The intensity of detected peaks in the Wild Yeast 2 sample was especially high compared to the other samples, so the PCA enabled identification of the characteristic compounds in Wild Yeast 2.

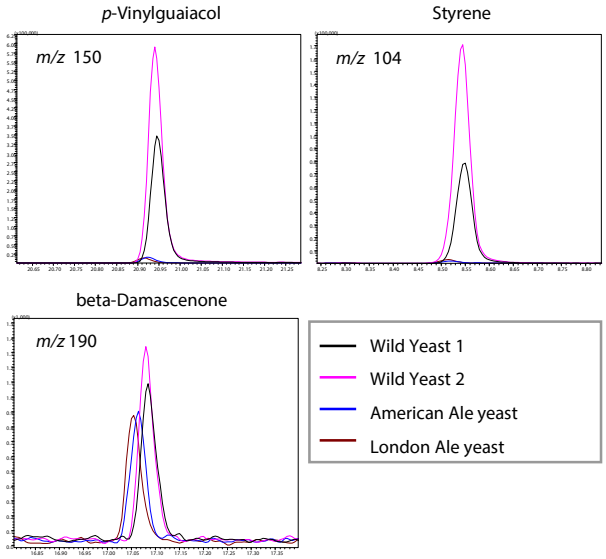


Fig. 7 Comparison of Mass Chromatograms of Characteristic Aroma Compounds in Wild Yeast 2

Furthermore, human sensory evaluation of the beer sample with the Wild Yeast 2 strain detected a spicy aroma. It was objectively confirmed by the PCA, which identified the compound *p*-vinylguaiacol, which is known to have a spicy aroma like cloves or curry.

In addition to analytical information, the Smart Aroma Database also contains sensory information. This enables simultaneous visual confirmation of sensory information and compound information, such as the retention time and area via the data analysis window (Fig. 8). These features enable the Smart Aroma Database to provide comprehensive support for the entire workflow, from aroma analysis to interpretation of analysis results.

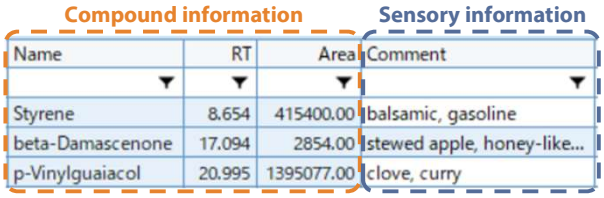


Fig. 8 Example of Analysis Using the Smart Aroma Database

■ Summary of Aroma Analysis Using GC-MS

In aroma analysis, wide target analysis was performed on four beer samples brewed with different yeasts using the Smart Aroma Database, and the characteristics of the resulting multivariate data were visualized by PCA.

In this analysis, PCA was used to compare the aroma compounds of the beers, and to determine the specific aroma characteristics of each beer. The results demonstrated the aromatic differences between each beer and how yeast strains influence the aromas of beers. Aroma analysis can thus be used to understand the effects of different yeast strains on beer aroma, and it can be applied to beer brewing processes that leverage these characteristics.

■ Targeted Metabolomics Using GC-MS and LC-MS

Metabolomics is the comprehensive analysis of the set of metabolites within a living organism that are known as the metabolome, and metabolomic techniques are used in various fields. In the food industry, they are used in quality control, manufacturing-storage process validation, and for the screening of functional compounds.

In metabolomic analysis, triple-quadrupole MS systems are ideally suited for use in targeted metabolomics. Techniques for analyzing metabolomic data using the Multi-omics Analysis Package[1)] are described below (Fig. 9). In this study, metabolomic data was obtained by performing GC-MS using the Smart Metabolites Database and LC-MS using the Method Package for Primary Metabolites Ver. 3 on the common samples. GC-MS typically involves a significant number of target compounds, and the need for trimethylsilyl (TMS) derivatization complicates the sample pretreatment process. On the other hand, while LC-MS is only capable of detecting a small number of compounds compared to GC-MS, some of these compounds can only be measured by it, and the sample pretreatment process is also very simple. Differential analysis of the GC-MS and LC-MS results was performed using the Multi-omics Analysis Package's multivariate analysis tools (EasyStats and Volcano Plot Generator). GC-MS and LC-MS data can also be integrated, and the results can be visualized on a metabolic map. For more information on metabolic maps, refer to the Application News edition that is listed in 2) in the references.

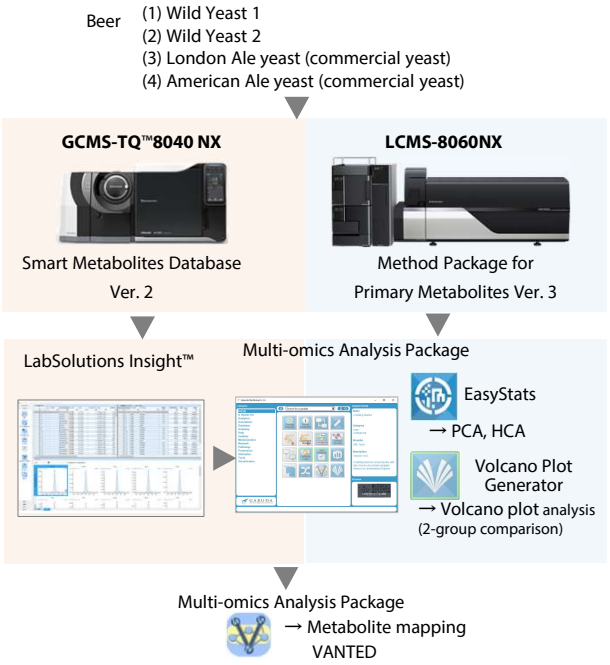


Fig. 9 Workflow of Metabolite Analysis

GC-MS Analysis
Deproteination/Extraction
Beer 100 µL
↓ Add 1.0 mL extraction solvent (a mixture of water, methanol and chloroform)
↓ Heat and agitate (37 °C, 30 min)
↓ Centrifuge (4 °C)
↓ Pipet 600 µL supernatant
↓ Add 300 µL water
↓ Centrifuge (4 °C)
↓ Pipet 200 µL supernatant
↓ Dry in centrifugal evaporator (2 hr)
↓ Freeze dry (overnight)

Derivatization (MeOx–TMS)
↓ Add 100 µL methoxy amine solution
↓ Heat and agitate (30 °C, 90 min)
↓ Add 50 µL MSTFA solution
↓ Heat and agitate (37 °C, 30 min)
↓ Centrifuge (room temp.)
Collect the supernatant into analysis vial

Fig. 10 Workflow of GC-MS Sample Pretreatment

■ Samples and Sample Pretreatment

The pretreatment of samples used in GC-MS is illustrated in Fig. 10. Following the extraction and deproteination of metabolites using an extraction solvent, the extracted metabolite solution was dried. Next, methoxymation and trimethylsilylation (MeOx-TMS) were performed, and the supernatant was used for analysis. Derivatization is not required in the pretreatment of LC-MS samples(Fig. 11). Deproteination was performed using an ultrafiltration membrane, and the filtrate was used for analysis. The samples contained high concentrations of citric acid, citrulline, guanosine, hypoxanthine, and phenylalanine, so the filtrate was diluted 100-fold with water, and results from the re-analyzed samples were used. Refer to the Pretreatment Procedure Handbook for Metabolite Analysis[3)] for a description of a typical pretreatment process.

LC-MS Analysis

Deproteination
↓ Centrifugal ultrafiltration filter
• Dispense into analysis vials (a water-diluted sample solution for analysis was also prepared)

Fig. 11 Workflow of LC-MS Sample Pretreatment

■ Analytical Conditions for Metabolite Analysis

Tables 2 and 3 show the analysis system and the analytical conditions used.

Table 2 GC-MS Analytical Conditions (Smart Metabolites Database Ver. 2)

GC-MS:	**GCMS-TQ8040 NX**
Auto-injector:	**AOC20i+s Plus**
GC	
Column:	DB-5 (30 m, 0.25 mm I.D., 1.00 µm)
Injection Temp.:	280 °C
Column Oven:	100 °C (4 min)→ 4 °C/min → 320 °C (8 min)
Injection Mode:	Splitless
Sampling Time:	1 min
Carrier Gas:	He
Carrier Gas Control:	Linear Velocity (39.0 cm/sec)
Injection Volume:	1 µL
MS	
Mode:	MRM
Ion Source Temp.:	200 °C
Interface Temp.:	280 °C

Table 3 LC-MS Analytical Conditions
(Method Package for Primary Metabolites Ver. 3)

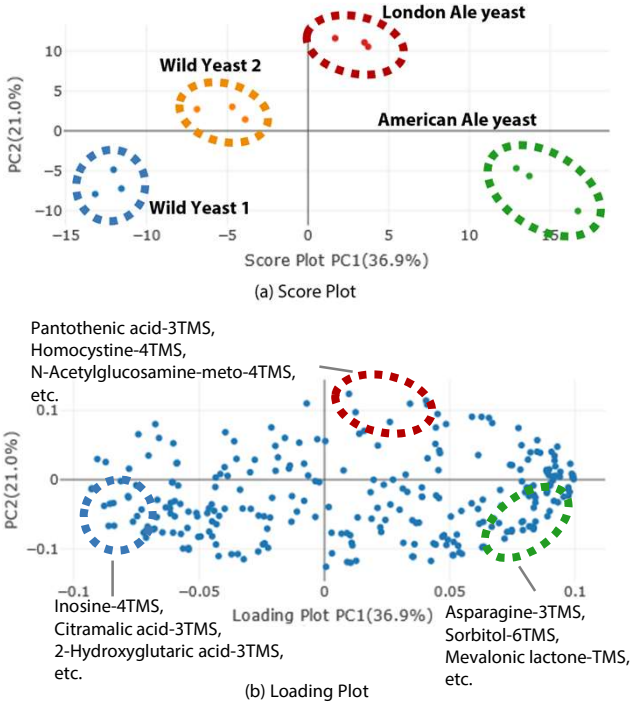
HPLC	**Nexera X3**
Column	Reversed-phase column
Column Oven	40 °C
Solvent A	0.1 % formic acid in water
Solvent B	0.1 % formic acid in acetonitrile
Mode	Gradient elution
Flowrate	0.25 mL/min
Injection Volume	3 µL
MS	**LCMS-8060NX**
Ionization:	ESI positive/negative (IonFocus™)
Mode:	MRM
Nebulizing Gas:	3.0 L/min
Drying Gas:	10.0 L/min
Heating Gas:	10.0 L/min
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
Interface Temp.:	270 °C

■ Results of Metabolite Analysis Using GC-MS

The GC-MS analysis of the metabolite compounds in the four beer samples resulted in the identification of 235 compounds. The detected areas were then corrected using the internal standard, and the area ratios were used in the PCA.

The results of the PCA are shown in Fig. 12. On the score plot in Fig. 12-(a), the four beer samples are separated into four clusters. The results resemble those of the aroma analysis, with the two commercial yeast beer samples plotted in the positive direction of PC1 and the two wild yeast beer samples plotted in the negative direction of PC1. The metabolite analysis results also demonstrated significant differences between the commercial and wild yeast strains.

The metabolite compounds with relatively high concentrations in each beer sample were confirmed via loading plot. Fig. 12-(b) shows the characteristic metabolite compounds present in each yeast strain.



(a) Score Plot

(b) Loading Plot

Fig. 12 Results of PCA of Metabolite Compounds Detected with GC-MS

Although the PCA differentiated each of the beer samples, when a sample was plotted close to the center of the score plot, as is the case for Wild Yeast 2, it proved difficult to identify its characteristic metabolite compounds from the loading plot. Therefore, in order to identify the characteristic metabolite compounds of the Wild Yeast 2 sample, a comparison of two groups was performed using Wild Yeast 2 as Group 1 and the other three yeast strains as Group 2. The compounds that served to differentiate these two groups were then visualized using a volcano plot (Fig. 13).

The results showed that the beer brewed with Wild Yeast 2 had a particularly high concentration of lactitol and maltose and a characteristically low concentration of some amino acids, such as isoleucine and valine.

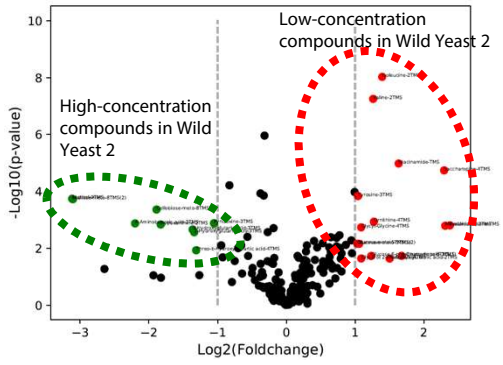
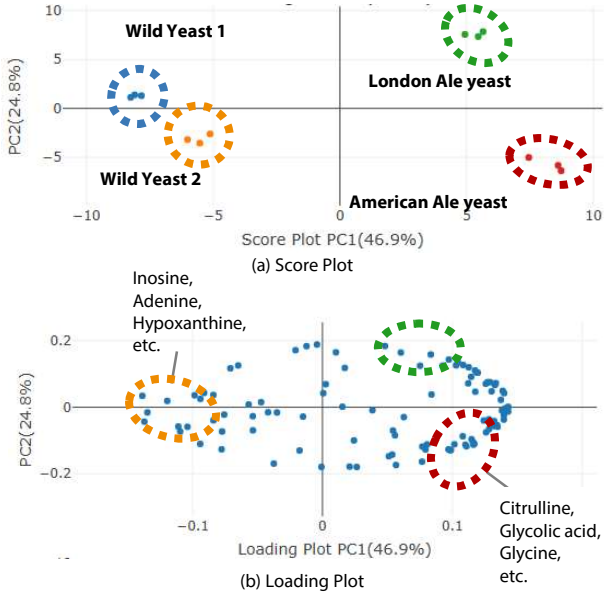


Fig. 13 Volcano Plot (GC-MS; Wild Yeast 2 Versus Others)

■ Results of Metabolite Analysis Using LC-MS

The four beer samples were analyzed using LC-MS. The Method Package for Primary Metabolites Ver. 3 provides two sets of analytical conditions. Based on the analytical conditions used in this study (Table 3), targeted analysis could be performed on 143 metabolite compounds. The peaks of 104 of these compounds were detected, and the calculated peak areas were used in multivariate analysis. The results of the PCA are shown in Fig. 14. In Fig. 14-(a), Wild Yeast 1 and 2 beer samples are plotted in the negative direction of PC1, while the London Ale and American Ale yeast beer samples are plotted in the positive direction. The London Ale and American Ale yeast beer samples are also separately plotted in the positive and negative directions of PC2, respectively.

The loading plot is shown in Fig. 14-(b). The results show that compounds such as inosine, hypoxanthine, and adenine were characteristically present in the wild yeast beer samples. While there was a high overall concentration of amino acids in the commercial yeast beer samples, there were particularly high concentrations of adenosine monophosphate, glutathione, and histidinol concentrations in the London Ale yeast sample and citrulline, glycolic acid, and glycine in the American Ale yeast sample.



(a) Score Plot

(b) Loading Plot

Fig. 14 Results of PCA of Metabolite Compounds Detected with LC-MS

The results of PCA in Fig. 14 show that both wild yeast strains are in close proximity, so their respective metabolic characteristics are difficult to distinguish on the loading plot. Therefore, the LC-MS results were used to generate a volcano plot comparing Wild Yeast 2 in Group 1 and Wild Yeast 1 in Group 2. The results of LC-MS analysis are shown in Fig. 15. Compared to the Wild Yeast 1 beer sample, the Wild Yeast 2 beer sample had high concentrations of proline and 2-aminobutyric acid and a low concentration of symmetric dimethylarginine.

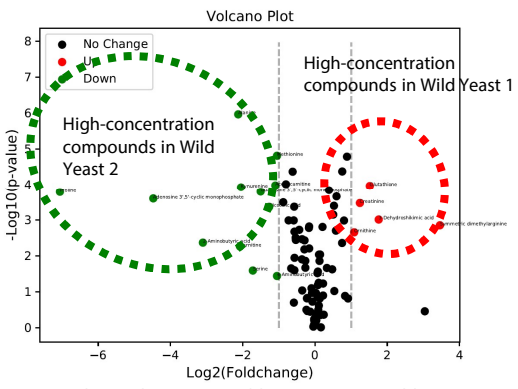


Fig. 15 Volcano Plot (LC-MS; Wild Yeast 2 Versus Wild Yeast 1)

■ Summary of Targeted Metabolomics Using GC-MS and LC-MS

The GC-MS metabolite analysis identified 235 compounds that consisted primarily of amino acids, organic acids, and sugars, while the LC-MS metabolite analysis identified 104 compounds that consisted primarily of amino acids, vitamins, and nucleobases. Excluding overlapping compounds, the combined GC-MS and LC-MS analyses identified a total of 278 metabolite compounds (Fig. 16).

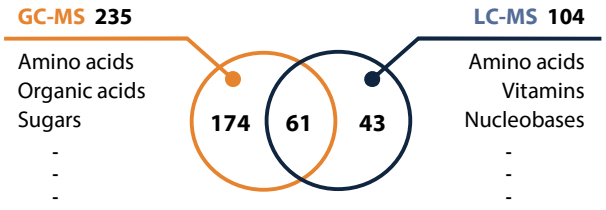


Fig. 16 Number of Metabolite Compounds Detected with GC-MS and LC-MS

Multivariate analysis of the metabolite compounds detected with each instrument confirmed the presence of compounds that were characteristic of the four types of beer samples. Principal component analysis separated wild and commercial yeasts on the first principal component axis in both GC-MS and LC-MS results. It shows that these features are significantly different. In addition, the datasets from both the GC-MS and LC-MS instruments also revealed a trend toward high inosine concentrations in the wild yeast beer samples. This demonstrates that the data from both instruments was consistent with each other.

On the other hand, citramalic acid and 2-hydroxyglutaric acid, which are characteristic compounds of wild yeast, were only detected by GC-MS, whereas adenosine monophosphate and glutathione, which are characteristic compounds of London Ale yeast, were only detected by LC-MS. In fact, there were many compounds that are characteristic of each beer that were only detected by one of the instruments. Given that the ability to easily detect a certain compound depends on the analytical technique being used, the use of both GC-MS and LC-MS enabled a more comprehensive assessment of the different characteristics of each yeast strain.

GC-MS analysis of the Wild Yeast 2 beer sample revealed characteristically low concentrations of the amino acids isoleucine and valine, which are consumed as nutrients during the yeast fermentation process (Fig. 17). This result led to the hypothesis that yeast actively consumes these amino acids during the fermentation process. From this, they were able to conceive of a brewing technique in which fermentation is promoted by increasing the proportion of ingredients containing nutrients required by yeast.

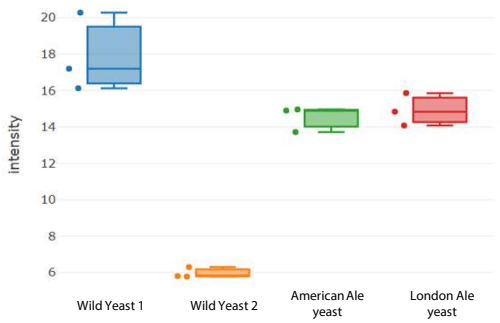


Fig. 17 Box Plot of Isoleucine Detected with GC-MS

■ Non-Targeted Metabolomics Using LC-QTOF

Targeted metabolomics is a simple analytical technique that targets only selected compounds but which may also overlook untargeted active ingredients. Conversely, non-targeted metabolomics comprehensively analyzes metabolites, including unknown compounds. Quadrupole time-of-flight (QTOF) mass spectrometer is suitable for use in non-targeted analysis as it can provide precise mass measurements, thus enabling putative identification of unknown compounds that differ between samples. In this study, Signpost MS multivariate analysis software (Reifycs Inc.) was used to perform differential analysis on the datasets of the four yeast strains of the beer samples. Signpost MS performs "spot sampling" to extract ion information from mass spectrometry data and then performs alignment, based on the retention time and mass-to-charge ratio (*m/z*). By performing multivariate analysis using aligned peaks, this software enables easy identification of the characteristic peaks of each sample. Shimadzu's QTOF qualitative analysis software LabSolutions Insight Explore™ is also an effective tool for attribution analysis of unknown compounds (Fig. 18).

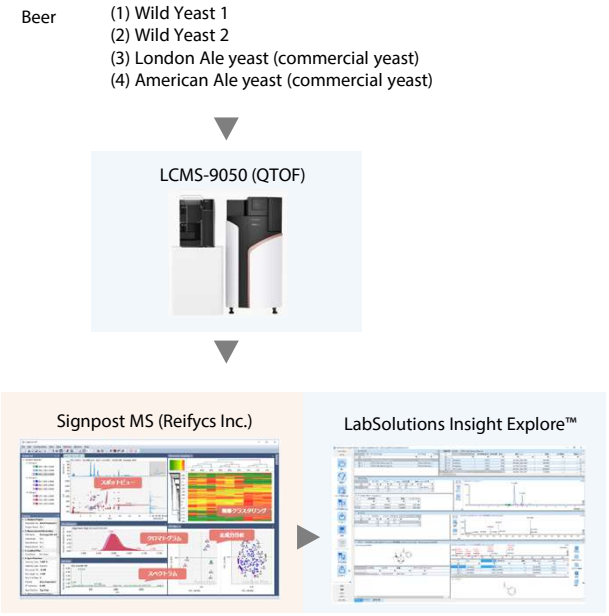


Fig. 18 Workflow of Non-Targeted Metabolomics

Table 4 Analytical Conditions

HPLC	**Nexera X3**
Column:	Reversed-phase column
Column Oven:	40 °C
Solvent A:	0.1 % formic acid in water
Solvent B:	0.1 % formic acid in acetonitrile
Mode:	Gradient elution
Flowrate:	0.25 mL/min
Injection Volume:	3 µL
MS	**LCMS-9050**
Ionization:	ESI negative
Mode:	Data Dependent Acquisition (DDA)
TOF-MS:	MS *m/z* 50-800 MS/MS *m/z* 10-800
Nebulizing Gas:	3.0 L/min
Drying Gas:	10.0 L/min
Heating Gas:	10.0 L/min
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
Interface Temp.:	300 °C

■ Sample Pretreatment and Analytical Conditions

As with the LC-MS targeted metabolomics procedure described above, deproteination was performed using an ultrafiltration membrane, and the filtrate was used for analysis. For the analysis, "Method Package for Primary Metabolites Ver. 3" for the triple quadrupole LC-MS was applied to the LCMS-9050. The HPLC and MS conditions are shown in Table 4.

■ Comprehensive Analysis of Metabolites in Beer

Each of the four beer samples were analyzed in triplicate using the LCMS-9050 system. A total of 4309 individual peaks were identified using Signpost MS software. The results of the PCA of the identified peak areas are shown in Fig. 19. Data scaling was performed using Pareto scaling. The loading plot is displayed with color-coding by using principal component variable grouping. The resulting data spots are grouped by relevance, thus simplifying interpretation of the principal components. On the score plot, the two wild yeast beer samples and the two commercial yeast beer samples are clustered separately along PC1. For illustrative purposes, the results of attribution analysis are presented below for the unknown compound (Compound A), which was found to be characteristic of the commercial yeast strains on the loading plot.

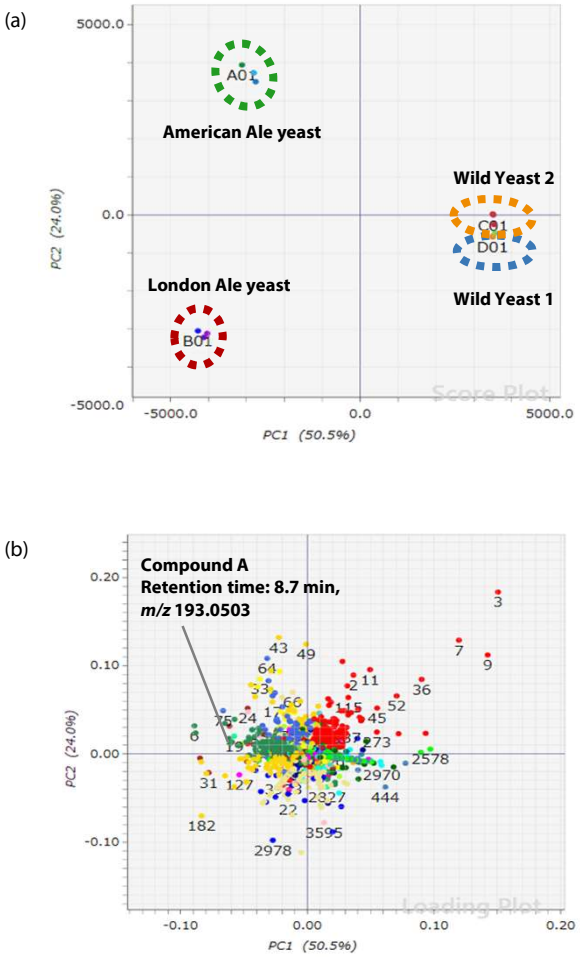
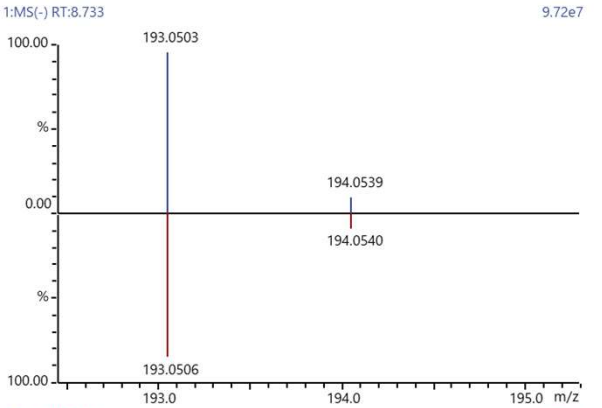


Fig. 19 Results of PCA Using Signpost MS
(a) Score Plot, (b) Loading Plot

■ Putative Composition

Composition was estimated based on the mass spectrum data using LabSolutions Insight Explore qualitative analysis software. Fig. 20 shows the putative composition of Compound A (retention time: 8.7 min, *m/z*: 193.0503), which was detected in the American Ale yeast beer sample using negative ion mode analysis. $C_{10}H_{10}O_4$ was proposed as the molecular formula candidate, based on a highly precise measurement of its mass with a mass error within ± 1 mDa.



#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
1	97.29	194.05791	193.05063	193.05031	-0.32	C10 H10 O4	[M-H]-	-1.658	97.92	6.0

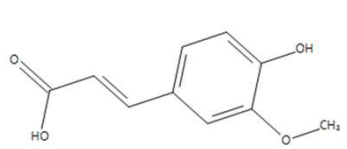
Fig. 20 Putative Composition of Compound A

Upper figure: Plot comparing analyzed mass spectra (upper blue lines) and theoretical mass spectra (lower red lines)

Lower figure: Molecular formula candidate

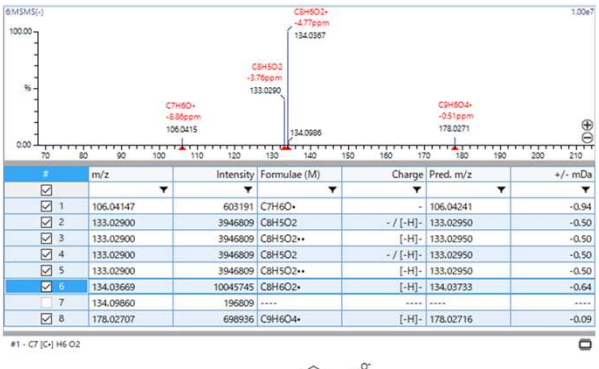
■ Compound Search and Fragment Attribution

An online search for the putative molecular formula was performed on the ChemSpider database using the assign function, and the top-ranked candidate compound was ferulic acid (Fig. 21). Fig. 22 shows the result of matching (i.e., fragment attribution) the predicted product ion, based on the structural formula and the product ion observed in the MS/MS spectrum.



#	Name	Formula	Mass	Assign Score (%)	#Reference	CSID
1	(E)-Ferulic acid	C10H10O4	194.05791	80.0	1223	393368
2	Dimethyl phthalate	C10H10O4	194.05791	80.0	291	13837329
3	(E)-Isoferulic acid	C10H10O4	194.05791	80.0	200	643318
4	Dimethyl terephth...	C10H10O4	194.05791	80.0	191	13863300
5	Methyl caffeate	C10H10O4	194.05791	80.0	176	600455
6	NT2540000	C10H10O4	194.05791	80.0	171	14360
7	Monoethyl phthal...	C10H10O4	194.05791	80.0	154	67856

Fig. 21 Results of ChemSpider Online Database Search for $C_{10}H_{10}O_4$ Molecular Formula



#	m/z	Intensity	Formulae (M)	Charge	Pred. m/z	+/- mDa
1	106.04147	603191	C7H6O•	-	106.04241	-0.94
2	133.02900	3946809	C8H5O2	- / [-H]-	133.02950	-0.50
3	133.02900	3946809	C8H5O2•-	[-H]-	133.02950	-0.50
4	133.02900	3946809	C8H5O2	- / [-H]-	133.02950	-0.50
5	133.02900	3946809	C8H5O2•-	[-H]-	133.02950	-0.50
6	134.03669	10045745	C8H6O2•	[-H]-	134.03733	-0.64
7	134.09860	196809	----	----	----	----
8	178.02707	698936	C9H6O4•	[-H]-	178.02716	-0.09

#1 - C7 [C•] H6 O2

m/z 134.0367
$C_8H_6O_2$

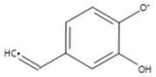


Fig. 22 Results of Automated Attribution of Compound A Fragment

■ Identification Using the Standard

The extracted ion chromatogram (EIC) of ferulic acid standard (*m/z* 193.0506) was compared against the EIC of Compound A, which was detected in the American Ale yeast beer sample (Fig. 23). The MS/MS spectral patterns of each compound were also compared (Fig. 24). The retention time and MS/MS spectral pattern of Compound A matched those of the standard, so it could be identified as ferulic acid.

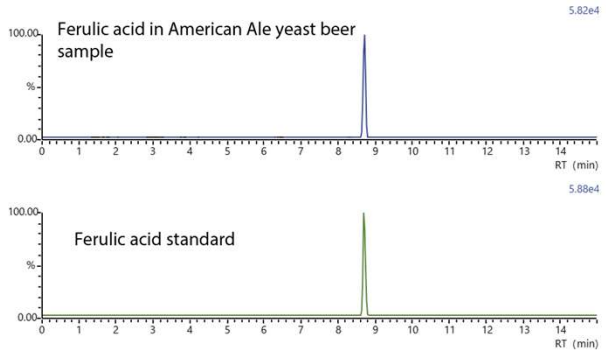


Fig. 23 Extracted Ion Chromatograms of Ferulic Acid

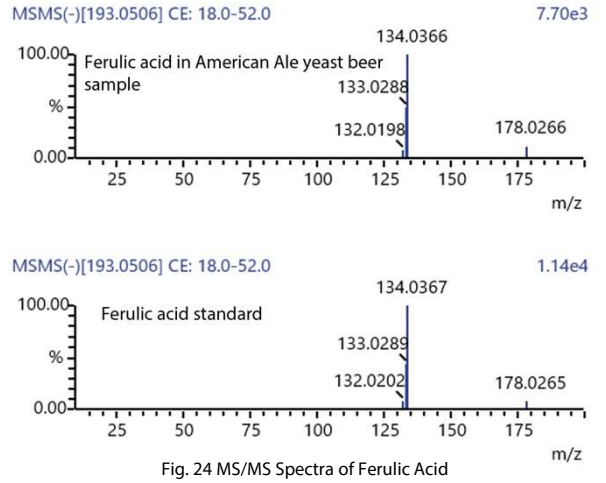


Fig. 24 MS/MS Spectra of Ferulic Acid

■ Summary of Non-Targeted Metabolomics Using LC-QTOF

QTOF-based precise mass analysis was performed on four beer samples that were brewed with different strains of yeast, and the characteristics of the resulting multivariate data were analyzed by PCA. Information on an unknown candidate compound was obtained by estimating the composition of the peak of the compound, which occurred in different quantities in the samples, conducting searches on an online chemical compound database, and performing fragment attribution analysis.

Based on the results of these analyses, the unknown compound that had a high concentration in the commercial yeast beer samples and a low concentration in the wild yeast beer samples was identified as ferulic acid. The spicy aroma of *p*-vinylguaiacol compound, which was identified by GC-MS aroma analysis as being present in the wild yeast beer samples, is reportedly synthesized by decarboxylation of ferulic acid in yeast. Therefore, it was thought that wild yeast strains produced a large amount of *p*-vinylguaiacol from ferulic acid.

■ Summary

In this study, beer was brewed using two strains of commercial yeast and two strains of naturally derived wild yeast. Comprehensive comparative analyses of the samples were then performed using various analytical instruments. In the aroma analysis that used GC-MS, the spicy aroma compound that is characteristic of wild yeast was identified. In the targeted metabolomic analysis using GC-MS and LC-MS, data was obtained on the metabolite compounds that were characteristic of each beer sample, and an approach to improve the brewing process was investigated. In the non-targeted metabolomic analysis using LC-QTOF, a comprehensive range of precise mass data was obtained, and a workflow that was used to identify the unknown compound was created.

■ Joint Development of a New Craft Beer

Sensory evaluation of Wild Yeast 2 beer revealed a spicy aroma. Through analysis, the principal component of this spicy aroma was identified as *p*-vinylguaiacol. A correlation between *p*-vinylguaiacol and ferulic acid, a substrate in biosynthesis, was also confirmed. The low concentrations of certain amino acids in the Wild Yeast 2 beer sample led to the hypothesis that the Wild Yeast 2 strain consumes a significant amount of amino acids and that it is possible to stabilize the fermentation process by adding wheat and oats. It was also confirmed that this sample had a high residual concentration of maltose that was derived from malt and that this maltose tended not to be consumed during the fermentation process. As beers with a high concentration of residual maltose tend to have a lingering aftertaste, this meant the aftertaste could be improved by adding sucrose. With this knowledge, Shimadzu was able to develop a Belgian IPA craft beer in collaboration with ISEKADO called Kocho (literally “aromatic harmony”) (Fig. 25). Using the Wild Yeast 2 strain, Shimadzu succeeded in imbuing the beer with a dry and balanced flavor while emphasizing the characteristic aroma of yeast.

Fig. 25 Jointly-Developed Craft Beer: KOCHO BREWED ON SCIENCE by ISEKADO and SHIMADZU Innovation 1

■ Acknowledgments

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