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Application News

No. M280

Gas Chromatography Mass Spectrometry

Investigation into Quality Evaluation Methods Involving Total Analysis of Metabolites in Beer

Although quality evaluation methods for food products vary greatly depending on the food type and investigative objectives, nowadays evaluations that examine food trends are widely conducted by performing total analysis followed by multivariate analysis on the components contained in the target food. In Application News No. M271, total analysis was performed on the aroma components and metabolites of Japanese rice wines using GC/MS followed by multivariate analysis of the obtained data in order to evaluate the quality of the rice wines.

In this article, metabolites contained in samples of different brands of beer are measured to investigate whether differences between brands of beer can be identified. Furthermore, since differences in taste among factories and production lots of alcoholic beverages such as beer are known to occur, this article also explores whether the differences in production factories and production lots can be identified within samples of the same brands of beer.

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Samples

Metabolites were extracted from each beer sample and derivatized before GC-MS analysis. A 10 µL aqueous solution of 2-isopropylmalic acid (0.5 mg/mL) was added as an internal standard substance to 50 µL of each sample. The solutions then underwent deproteinization, hydrophilic metabolites were extracted, and these were thoroughly exsiccated using a centrifugal concentrator. An 80 µL methoxyamine hydrochloride/pyridine solution (20 mg/mL) was added to the remnants after exsiccation and the solutions were shaken for 90 minutes at 30 °C. Next, 40 µL of N–Methyl-N–(trimethylsilyl) trifluoroacetamide (MSTFA) was added and the solutions were shaken for 30 minutes at 37 °C. Finally, the solutions were put into GC-MS vials and analyzed. Table 1 lists the analyzed beer samples.

Table 1 Beer Samples Used in Each Analysis

Beer Sample	Analysis 1 Comparison of Brand Differences	Analysis 2 Comparison of Factory/lot Differences
Lager beer A (Factory a)	✓	✓
Lager beer A (Factory b)		✓
Lager beer A (Factory c)		✓
Pale ale A (Factory a)	✓	✓
Pale ale A (Factory b, lot a)		✓
Pale ale A (Factory b, lot b)		✓
Pale ale A (Factory b, lot c)		✓
Pale ale B (Factory a, lot a)	✓	✓
Pale ale B (Factory a, lot b)		✓
Pale ale B (Factory a, lot c)		✓
Pale ale C	✓	
IPA beer A	✓	

Analysis Conditions

lists the configuration of the instrument used for analysis and the analysis conditions.

Table 2 Instrument Configuration and Analysis Conditions

Instrument	:	GCMS-TQ™8040 triple quadrupole gas chromatograph mass spectrometer
Option software	:	Smart Metabolites Database™
GC		
GC column	:	DB-5 (30 m × 0.25 mm I.D., 1.00 µm)
Carrier gas	:	He
Vaporizing chamber temperature	:	280 °C
Control mode	:	Linear velocity (39.0 cm/s)
Injection method	:	Splitless
Sampling time	:	1 min
Purge flow rate	:	5.0 mL/min
Oven temperature	:	100 °C (4 min) → (10 °C/min) → 320 °C (11 min)
MS (EI method)		
Ion source temperature	:	200 °C
Interface temperature	:	280 °C
Tuning mode	:	Standard
Measurement mode	:	MRM
Loop time	:	0.25 s

Analysis

Multivariate analysis was performed on the measurement results of GC-MS analysis using the SIMCA® 15 multivariate analysis software (INFOCOM CORPORATION).

Results of Analysis 1 (Comparison of Brand Differences)

Peak identification was performed on the analysis results based on the quantitation/confirmation ions and retention indices of compounds registered in the Smart Metabolites Database. Main component analysis was performed using the 300 components detected in all samples in this analysis. Fig. 1 shows a score plot of the results.

The five samples were significantly separated on the score plot. Fig. 2 shows the corresponding loading plot. Table 3 lists the components identified from the loading plot that were contained in each beer in relatively high quantities. The loading plot shows that IPA beer A and pale ale C contained relatively higher quantities of certain saccharides than the other beers.

Image of External Appearance of GCMS-TQ8040 NX

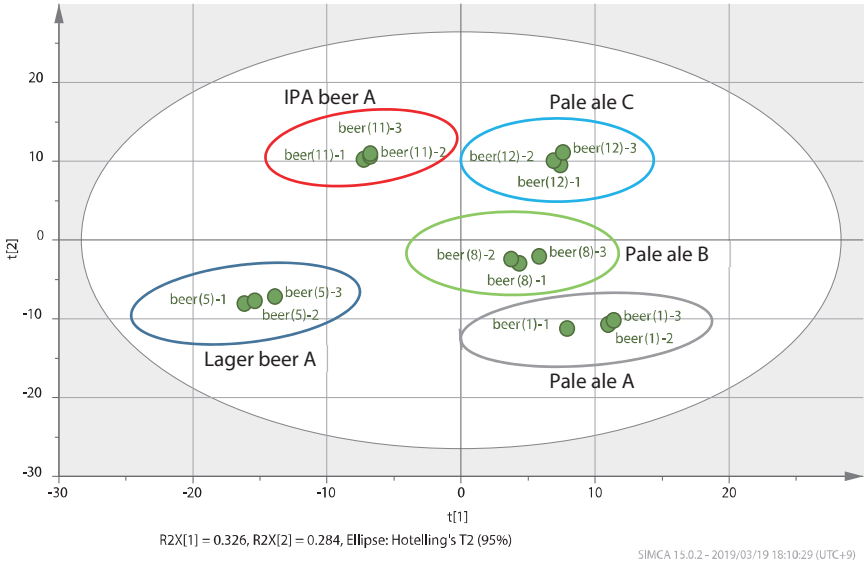

Fig. 1 Score Plot of Brand Differences

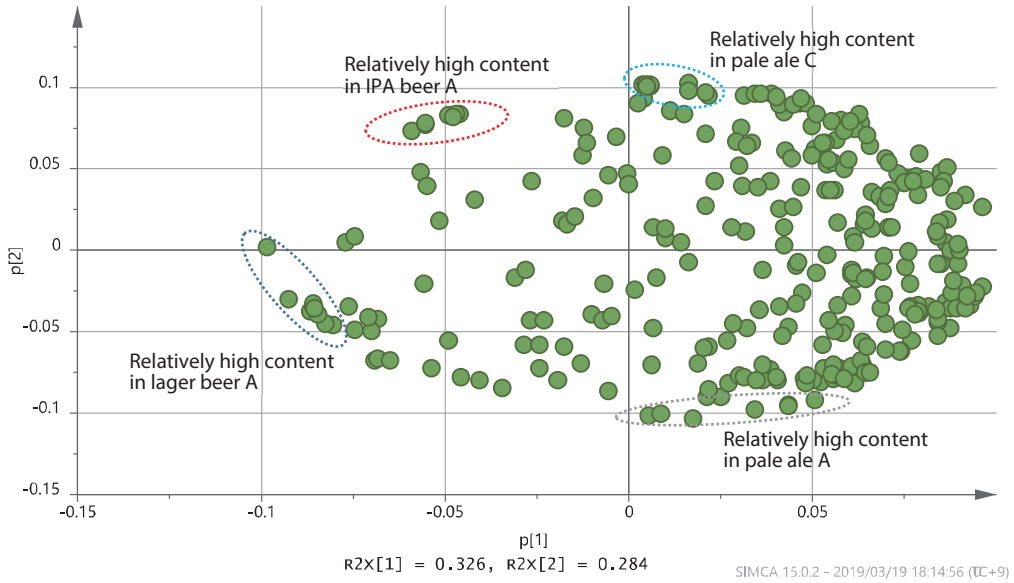

Fig. 2 Loading Plot of Brand Differences

Table 3 Components in Relatively High Quantities in Each Beer

Beer Sample	Component in Relatively High Quantity in Each Beer	Beer Sample	Component in Relatively High Quantity in Each Beer
Lager beer A	Phenylpyruvic acid Glutaric acid Lyxose Xylose Arabinose Threo-b-hydroxyaspartic acid 2-Ketoglutaric acid 2-Hydroxyglutaric acid	Pale ale C	Galactose Galacturonic acid Glucose Mannose Erythrulose Homogentisic acid Glucuronic acid Asparagine
Pale ale A	Maleic acid Cadaverine Maltitol 4-Aminobutyric acid Dopamine Tryptophan Oxalic acid	IPA beer A	Sebacic acid Fructose Sorbose Tagatose Psicose

Results of Analysis 2 (Comparison of Factory/Lot Differences)

Peak identification and main component analysis were performed using the same method as analysis 1. Fig. 3 shows a score plot of the results. The three brands of beer were significantly separated on the score plot. Furthermore, the plot shows separation among the beers of the three factories producing lager beer A. There was separation between factory a and factory b for pale ale A, and for factory b there was also separation among the three beers produced in different lots. There was no significant separation among different lots for pale ale B. Next, hierarchical cluster analysis was performed in order to visualize similarities and Fig. 4 shows the resulting tree diagram. The differences among the individual beers were even more discernible in the tree diagram.

These results allowed visualization of the differences in quality between factories and lots and hinted at possibilities of using such results as objective indicators of quality differences and for quality adjustments.

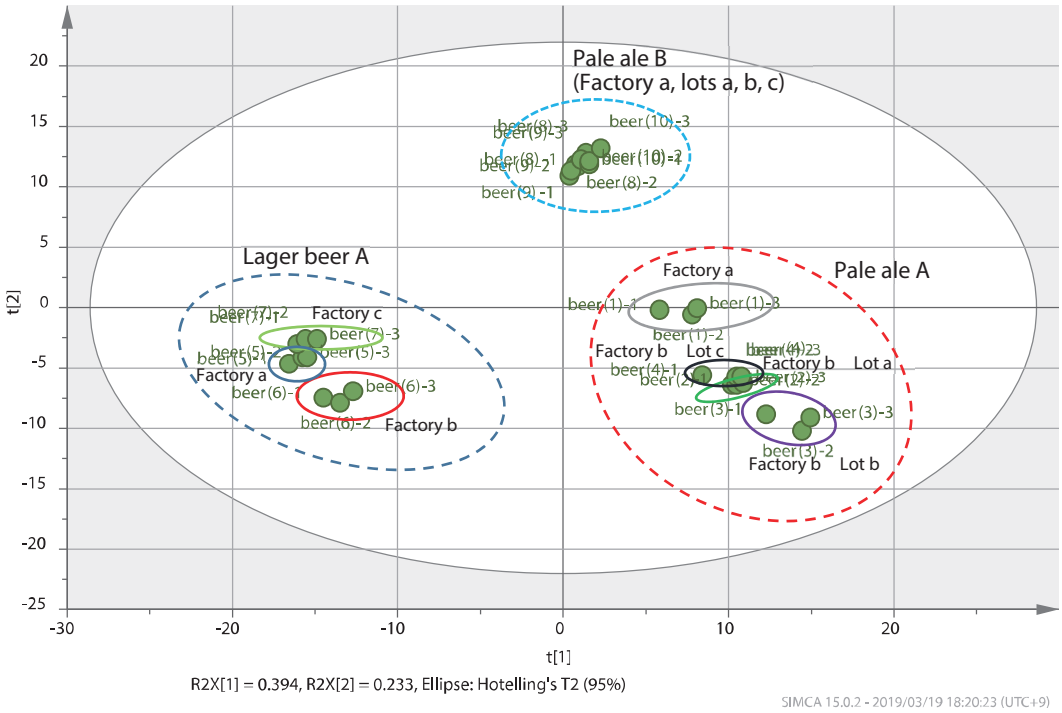

Fig. 3 Score Plot of Factory and Lot Differences Among Three Brands

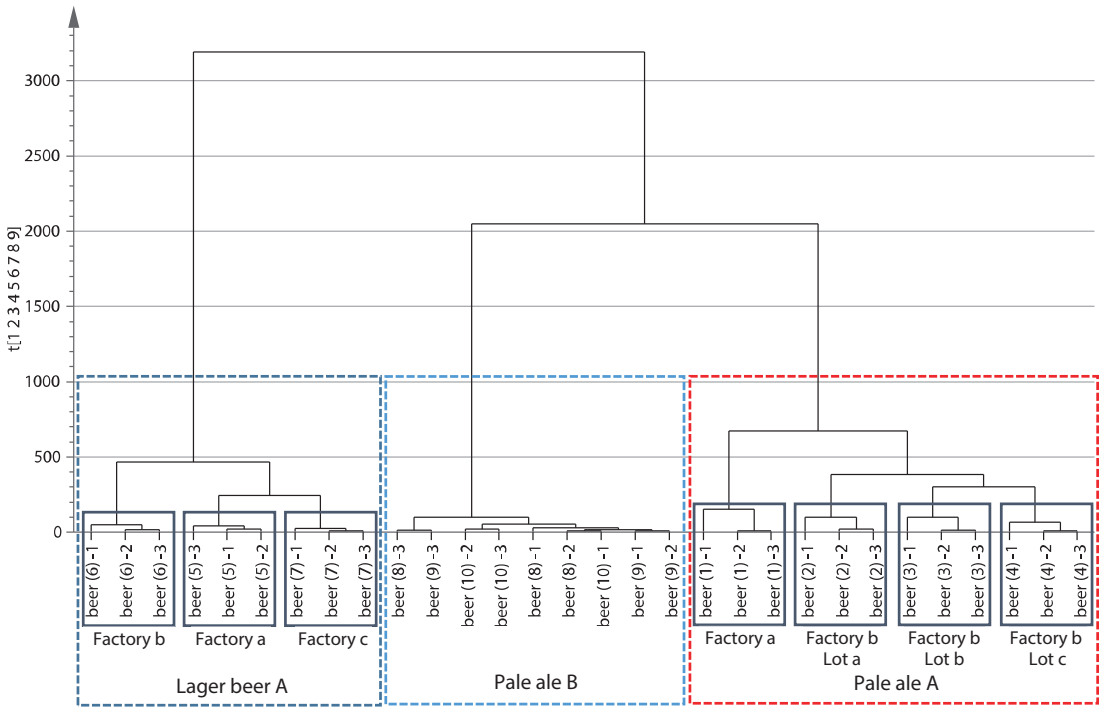

Fig. 4 Tree Diagram of Factory and Lot Differences Among Three Brands

Main component analysis was also performed on pale ale A alone in order to determine the types of components where quality differences were occurring in different factories and different lots for beer of the same brand. Fig. 5 shows the results on a score plot and Fig. 6 shows the results on a loading plot. Table 4 lists the components identified from the loading plot that were contained in each beer in relatively high quantities. These results show that beer produced at factory a has relatively higher quantities of metabolites of certain saccharides than factory b.

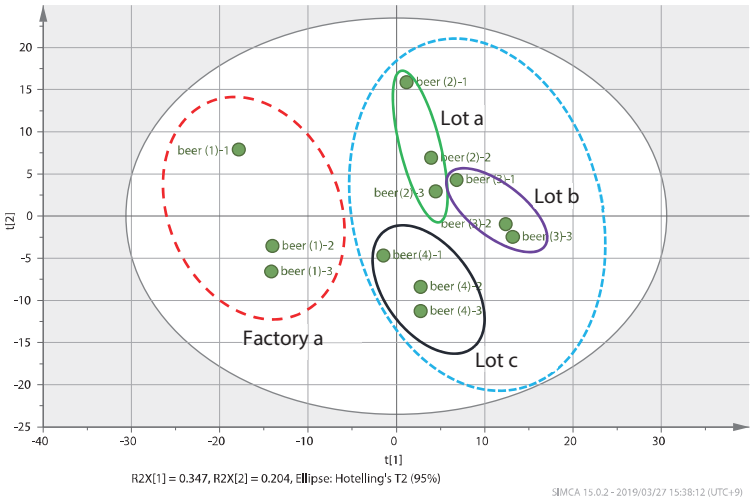

Fig. 5 Score Plot of Pale Ale A

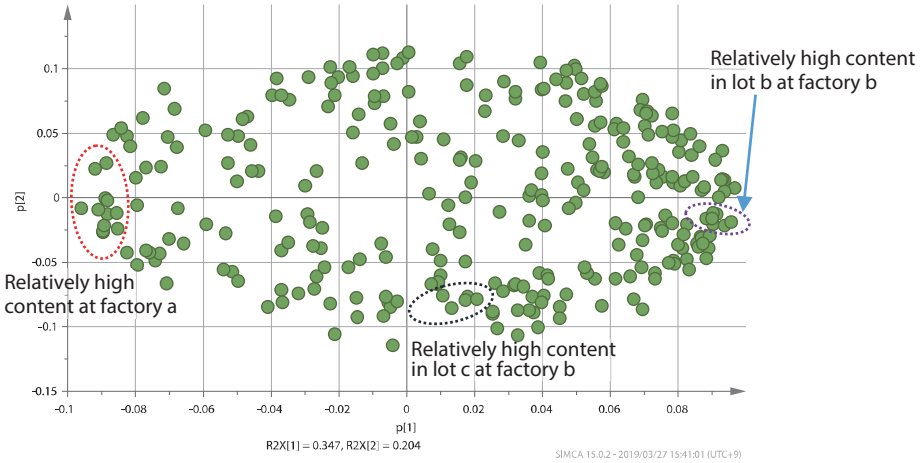

Fig. 6 Loading Plot of Pale Ale A

Table 4 Components in Relatively High Quantities in Each Beer

Beer Sample	Component in Relatively High Quantity in Each Beer	Beer Sample	Component in Relatively High Quantity in Each Beer
Factory a	3-Phenyllactic acid, Trehalose, Glyceric acid, Fructose 1-phosphate, Nonanoic acid 2-Hydroxyisobutyric acid, Caproic acid Glucose 6-phosphate, Sedoheptulose 7-phosphate Mannose 6-phosphate, Glucose 6-phosphate	Factory b	Allose, Lysine, Tyramine, Methionine Glutamic acid, Galactose Phenylpyruvic acid, Tryptamine 2'-Deoxyuridine, Cystamine-d8 Uridine

Conclusion

Differences among individual brands of beer were successfully identified by measuring and performing multivariate analysis on the metabolites in samples of several brands of beer. A high content of metabolites in each brand of beer was also confirmed. Quality differences were visualized by using the same measurement and analysis method on samples of beer from different production factories and production lots of the same brand. This allowed identification of important components that are responsible for quality differences.

<Acknowledgments>
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