# SHIMADZU

# Metabolomic Profiling of Beer Using GC-MS and GC-FID

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## 1. Overview

Despite the same brand and identical brewing techniques, it is widely known that beer taste and quality is not consistent from plant to plant. Therefore, brewers normally check beer quality mainly by sensory test method, and in turn, adjust beer taste in order to reduce these differences as much as possible. In this poster, by metabolomics profiling using GC-MS and GC-FID, we introduce a new approach towards classification and visualization of beer quality to identify how specific components influence taste from plant to plant.

## 2. Introduction

In food science, global metabolite analysis, or 'metabolomics', is increasingly applied to a number of value-added food production areas including food-safety assessment, quality control, food authenticity, origin and processing. GC-MS are widely used for measuring the total amount of metabolites in a sample. Whereas GC-FID might be less utilized in Metabolomics than GC-MS so far. This is because GC-MS is more powerful in peak annotation, and peak annotation is considered necessary for Metabolomics. But GC-FID has a massive feature; low cost, easy to use, and better reputability.



**GC-MS(/MS)**
- Permits comprehensive measurement of several hundred compounds in a single measurement
- Better peak annotation
- First-choice comprehensive measurement

**GC**
- Simple measurement
- Ideal for efficient routine measurement of specific compounds
- Better repeatability
- Low cost

Figure1  Instrument Features

In order to introduce a new approach towards classification and visualization of beer quality, we tried doing the following two studies.

Study1: Classification of different brand beers
Five brand beers were analyzed by GC-FID and GC-MS, and then principal component analysis (PCA) was performed using each GC-FID and GC-MS data. The results of PCA from each GC-FID and GC-MS data were compared. From loading plot of GC-MS data, we can successfully identify how specific components influence this classification.

Study2: Classification of same brand beers
The different plant and lot beers in the same brand were analyzed by GC-FID and GC-MS, and then principal component analysis (PCA) was performed using each GC-FID and GC-MS data. The results of PCA from each GC-FID and GC-MS data were compared. From loading plot of GC-MS data, we can successfully identify how specific components influence this classification.

## 3. Methods

### 3-1. Samples

After metabolites were extracted from beers, they were derivatized. Table 1 shows pretreatment procedure for the sample of  GC-MS and GC-FID, and Table 2 shows types of  analyzed beer  samples.

Table1 Pretreatment procedure

50µL degassed beers
↓
An internal standard※ was added.
↓
Extraction of hydrophilic metabolites.
↓
Drying
↓
Samples were methoximated and trimethylsilylated.

※ 2-Isopropylmalic acid  (0.5 mg/mL)

Table2  Types of  analyzed beer  samples

| Types of beer | Study1 | Study2 |
|---|---|---|
| Lager A | o | |
| Pale ale A（Plant: a） | o | o |
| Pale ale A（Plant: b, Lot: a） | | o |
| Pale ale A（Plant: b, Lot: b） | | o |
| Pale ale A（Plant: b, Lot: c） | | o |
| Pale ale B | o | |
| Pale ale C | o | |
| IPA A | o | |

### 3-2. Analytical conditions and software

Pretreated beers were analyzed by GC-MS triple quadrupole mass spectrometer and GC-FID. For statistical analysis, SIMCA 15  software (INFOCOM CORPORATION) was used.

Table3 Analytical condition in GC-MS/MS

**GC-MS/MS : GCMS-TQ8040**
Software :
  Smart Metabolites Database (475 compounds)
**GC conditions**
  Column: DB-5（30 m ×0.25 mm I.D., 1.00 µm）
  Carrier Gas: He
  Injection Temperature : 280 ° C
  Control Mode : Linear velocity (39.0 cm/sec)
  Injection Method : Splitless
  Sampling time: 1min
  Oven Temperature :
    100° C (4 min) - 10 ° C/min - 320 ° C (11 min)
**MS conditions**
  Ion Source Temperature : 200 ° C
  Interface Temperature : 280 ° C
  Tuning Mode : Standard
  Measurement Mode : MRM
  Loop Time : 0.25 seconds
  MRM Transitions : 950 Transitions

Table4 Analytical condition in GC

**GC : GC-2030**
  Column: SH-Rtx-1（60 m ×0.32 mm I.D., 1.00 µm）
  Carrier Gas: He
  Injection Temperature : 280 ° C
  Control Mode : Linear velocity (25.0 cm/sec)
  Injection Method : Split (1:15)
  Oven Temperature :
    40° C - 4 ° C/min - 320 ° C (15min)
  Detector : FID
  Detector Temperature : 330 ° C

## 4. Results

Principal component analyses (PCA) for GC-MS/MS and GC-FID data were performed in the following study 1 and 2.

### 4-1. Study1: Classification of different brand beers

**Principal component analyses for GC-MS/MS**
Figure2 shows Score plot and loading plot from GC-MS/MS data. From Score plot of figure2, we can confirm that five types of beers were successfully classified. Loading plot of figure2 means which compounds are relatively higher concentration among five beers and how specific compounds influence this classification. Table5 shows higher concentration compounds in each beer from loading plot. In IPA A and Pale ale C, a lot of some sugars were remained.

**Principal component analyses for GC-FID**
Figure3 shows the score plot from GC-FID data. We can get almost the same score plot as one of GC-MS/MS. This result suggest that  GC-FID can also used for classification of beers quality. Whereas, it is difficult  how specific compounds influence this classification from only FID data, because qualitative analyses performance on GC-FID  is less than GC-MS.





Figure3  Score plot from GC-FID data



Figure2  Score plot and loading plot from GC-MS/MS data

Table5  Higher concentration compounds in each beer from loading plot

| Types of beer | Higher concentration compounds |
|---|---|
| Lager A | Phenylpyruvic acid,  Glutaric acid  Lyxose,  Xylose,  Arabinose  Threo-b-hydroxyaspartic acid  2-Ketoglutaric acid  2-Hydroxyglutaric acid |
| Pale ale A | Maleic acid,  Cadaverine,  Maltitol  4-Aminobutyric acid,  Dopamine  Tryptophan,  Oxalic acid |
| Pale ale C | Galactose,  Galacturonic acid  Glucose,  Mannose,  Erythrulose  Homogentisic acid,  Glucuronic acid  Asparagine |
| IPA A | Sebacic acid,  Fructose,  Sorbose  Tagatose, Psicose |

## 4-2. Study2: : Classification of same brand beers

**Principal component analyses for GC-MS/MS**
Figure4 shows score plot and loading plot from GC-MS/MS data. From Score plot of figure4, we can confirm that the same brand beers brewed in different plants were divided. Plus, different lots of same plant can successfully classified in the score plot. Loading plot of figure4 means which compounds are relatively higher concentration among  the same brand beers and how specific compounds influence this classification. Table6 shows higher concentration compounds in beers brewed in Plant a and b from loading plot. In the beer brewed in Plant a, a lot of metabolites from sugar were remained. These results suggest that  this approach could be a powerful tool for adjusting food quality.

**Principal component analyses for GC-FID**
Figure4 shows the score plot from GC-FID data. We can get almost the same score plot as one of GC-MS/MS. This result suggests that GC-FID can also used for classification of beers quality in same bland.



Figure4  Score plot from GC-FID data



Figure4  Score plot and loading plot from GC-MS/MS data

Table6  Higher concentration compounds in each plant beer from loading plot

| Types of plant | Higher concentration compounds |
|---|---|
| Plant 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 |
| Plant b | Allose,  Lysine,  Tyramine,  Methionine  Glutamic acid,  Galactose  Phenylpyruvic acid,  Tryptamine  2'-Deoxyuridine,  Cystamine-d8,  Uridine |

## 5. Conclusions

・ Score plot from GC-MS/MS and GC-FID successfully categorized five brands of beer, different plants and lots in same brand beer.
・ From the loading plots in GC-MS/MS, we could  identify important compounds which determine differences of beer brands, same brand beers brewed in different plants and product lots.
・ This study suggest a new approach towards classification and visualization of beer quality.
・ In the future,  GC-FID potential for this filed will be tested in more detail.

## 6. Acknowledgment