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Application News

No. M311A

Gas Chromatography Mass Spectrophotometry GCMS™-2020 NX

Quality Evaluation of Food Products Using Multivariate Analysis (2): Analysis of Aromatic Components in Tomato Juice

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User Benefits

- High sensitivity analysis of samples containing moisture is possible by concentration using the trap model of HS-20 headspace sampler.
- Objective evaluation of components with differences between samples is possible by multivariate analysis.
- All process from deconvolution to multivariate analysis are completed with one software program when using AnalyzerPro®.

■ Introduction

It is known that taste, texture, and flavor are important elements of the "deliciousness" of food. In the recent food and beverage markets, differentiation from other products has been promoted through product development with the aims of higher quality and higher functionality.

Breeding and selection of food materials is one method for achieving differentiation. For example, it is known that the genetic differences between farm products influence differences in their aromatic components. Vegetable and fruit drinks are also marketed as foods with health claims which do not contain flavor additives, heightening the need for evaluation of differences in the materials (products) themselves.

In Application News No. M310, a multivariate analysis in the metabolites in three types of tomato juice was carried out, and the differences in the components contributing to taste and functional components between the products were introduced.

In this article, an analysis of aromatic components by the headspace (HS) method was conducted using the same three commercially-available tomato juices as in No. M310, and deconvolution and multivariate analysis were conducted using the AnalyzerPro software (SpectralWorks Ltd.), as introduced here.

■ Sample Preparation and Analysis Conditions

Three types of commercially-available tomato juice were used as samples. These products were selected because raw their materials used in production were limited to only tomatoes or tomatoes and common salt (table salt).

A Shimadzu headspace (HS) gas sampler was used to capture the aromatic components. The trap model of the HS-20 headspace gas sampler series features a built-in electronic cooling trap and enables high sensitivity analysis by concentrating aromatic components. Samples containing moisture can also be analyzed by concentrating low boiling-point compounds to high boiling-point compounds.

Analysis samples were prepared by taking 5 mL of the tomato juice in a 20 mL headspace vial and adding 10 µL of a 10 µg/mL solution of p-bromofluorobenzene (methanol) as an internal standard. Table 1 shows the measurement conditions.

Table 1 Measurement Conditions

Headspace gas sampler	: HS-20
GC-MS	: GCMS-QP™2020 NX
Column	: SH-PolarWax (length 30 m, 0.32 mm I.D., df = 0.50 µm) *1
[HS]	
Mode	: Trap
Trap tube	: Tenax® TA
Oven temp.	: 40 °C
Sample line temp.	: 90 °C
Transfer line temp.	: 150 °C
Trap cooling temp.	: 0 °C
Trap heating temp.	: 220 °C
Trap waiting temp.	: 0 °C
Vial agitation	: 2 times
Multi-injection	: 5 times
Vial pressurizing gas pressure	: 100 kPa
Dry purge gas pressure	: 20 kPa
Vial heat-retention time	: 10 min
Vial pressurization time	: 0.5 min
Pressure equilibration time	: 0.1 min
Load time	: 0.5 min
Load equilibration time	: 0.1 min
Dry purge time	: 2 min
Injection time	: 3 min
Needle flush time	: 5 min
Sample injection volume	: 5 mL
[GC]	
Carrier gas	: He
Carrier gas control	: Linear velocity (45.0 cm/s)
Injection mode	: Split
Split ratio	: 10
Oven temp.	: 40 °C (2 min) →(5 °C/min) →200 °C (1 min)
[MS]	
Ion source temp.	: 200 °C
Interface temp.	: 230 °C
Ionization mode	: EI
Measurement mode	: Scan (*m/z* 35 - 400)
Event time	: 0.3 s

*1 P/N: 227-36251-01

■ Results

Fig. 1 shows the TICCs (total ion current chromatograms) of the tomato juices. The substances detected in common in all three samples included aldehydes (acetaldehyde, pentanal, furfural, etc.), sulfides (dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide), furans (furan, 2-methylfuran, 2-pentylfuran, etc.), ketones (2-butanone, etc.), and terpenes (d-limonene, p-cymene, etc.), among others.

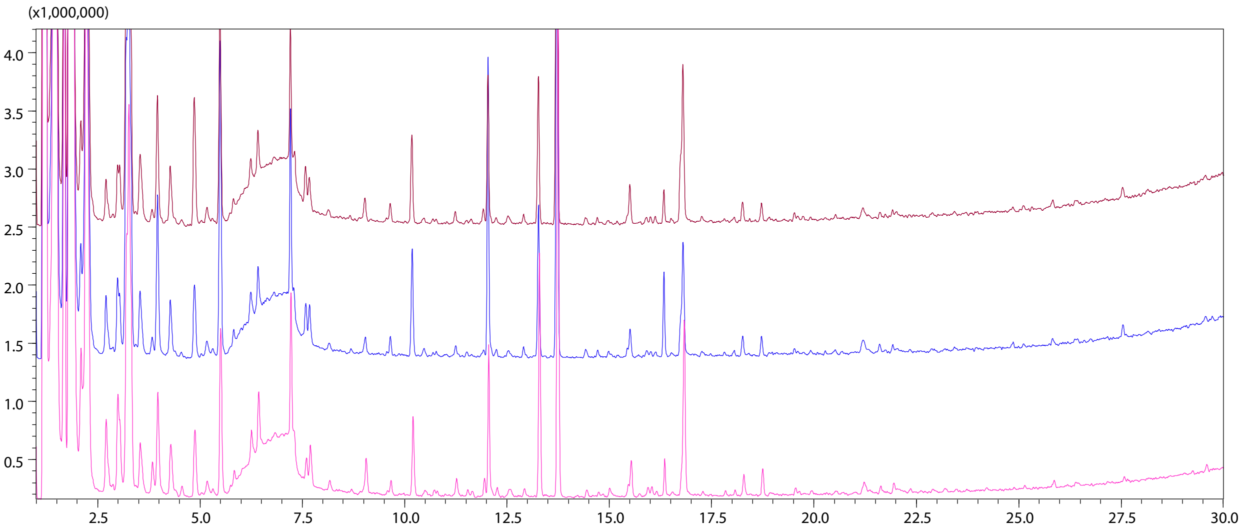


Fig. 1 Analysis Results of Tomato Juices (TICC)
Brown: Sample 1, Blue: Sample 2, Pink: Sample 3

■ Multivariate Analysis

AnalyzerPro (SpectralWorks Ltd.) is a software program that can carry out the entire series of processes from direct reading of the data acquired by a Shimadzu GC-MS, deconvolution, peak detection, library search, alignment and compare the differences between samples. In multivariate analyses, it can conduct principal component analysis (PCA) and volcano plots for two-group comparisons. Fig. 2 shows the analysis window of AnalyzerPro.

In the analysis by AnalyzerPro, data acquired 3 times for each sample were used, and the intensities of the detected compounds were normalized by using p-bromofluorobenzene as an internal standard. The tomato juices used here were labeled Samples 1 to 3. Only Sample 1 contained added table salt, and Sample 3 was made from the high-grade variety of tomato.

A principal component analysis (PCA) was conducted using the results obtained from the three tomato juices. In this PCA, the analysis was carried out by the use of components invariably detected in at least one sample. Fig. 3 and Fig. 4 show the results of the score plot and loading plot of the PCA, respectively. The first principal component (PCA 1, abscissa) is 58.5 %, and the second principal component (PCA 2, ordinate) is 16.2 %. Thus, the samples were clearly separated, confirming that the respective samples can be distinguished.

The components with positive values of PCA 1 in the loading plot tended to have large contents in Sample 3, while those with negative values of PCA 1 tended to have large contents in Samples 1 and 2. Focusing on the positive components, it was suggested that Sample 3 tends to have larger contents of many components such as furans and terpenes than the other two samples. Among the negative components, benzyl nitrile and others were detected.

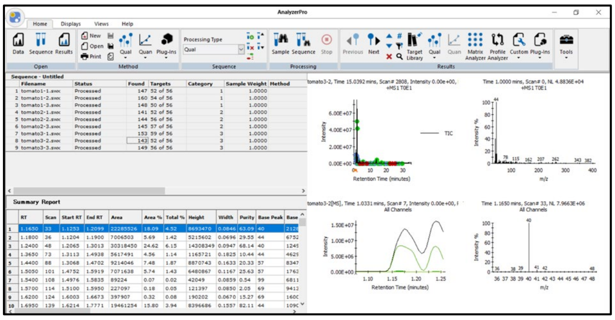


Fig. 2 Analysis Window of AnalyzerPro (Ver. 6.0)

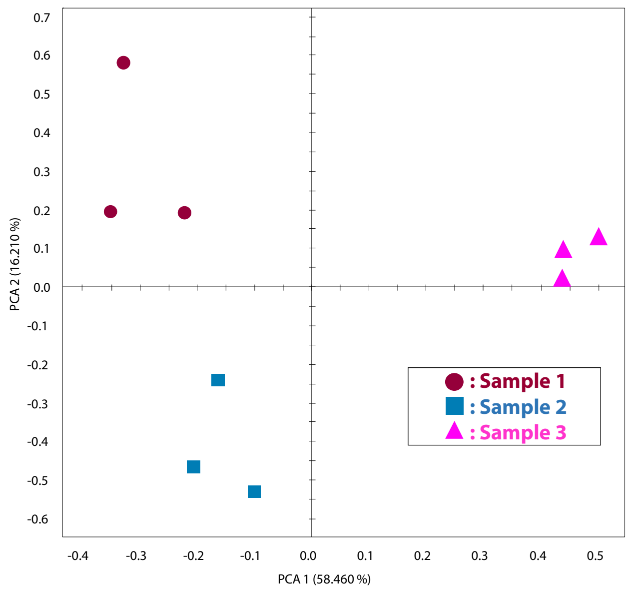


Fig. 3 Principal Component Analysis (PCA)
Score Plot

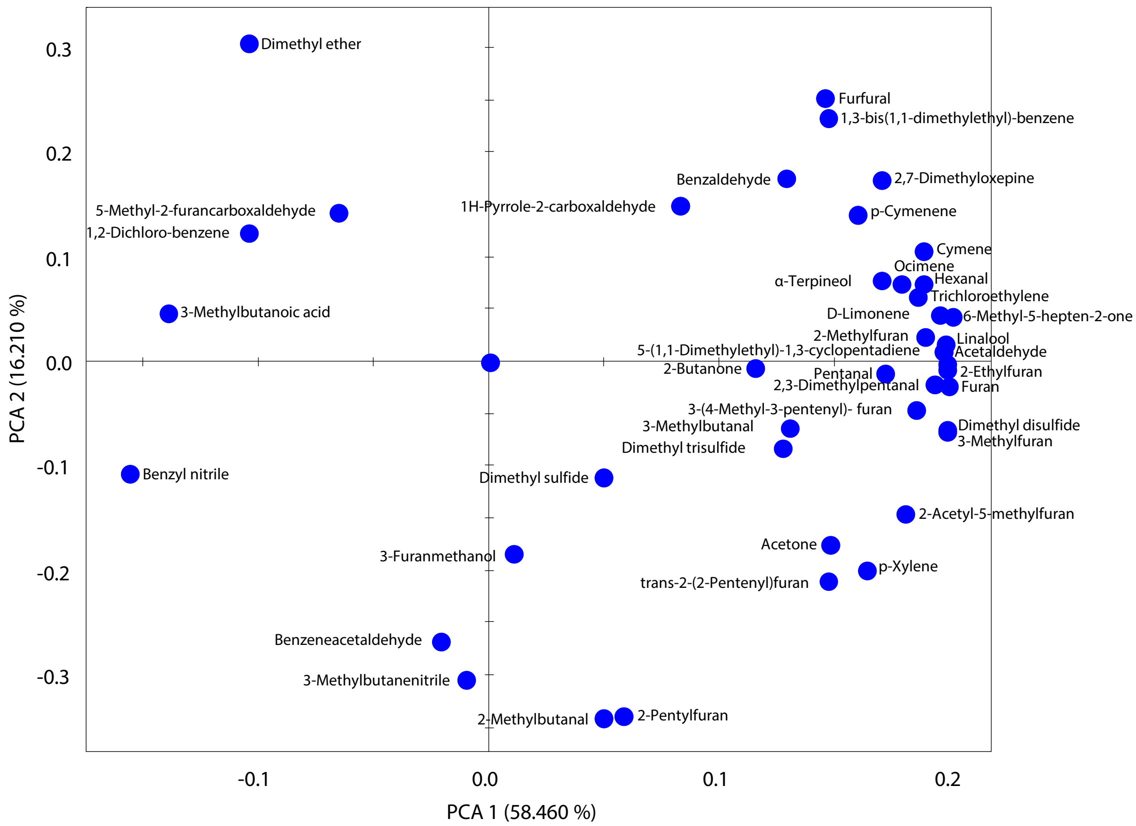


Fig. 4 Principal Component Analysis (PCA)
Loading Plot

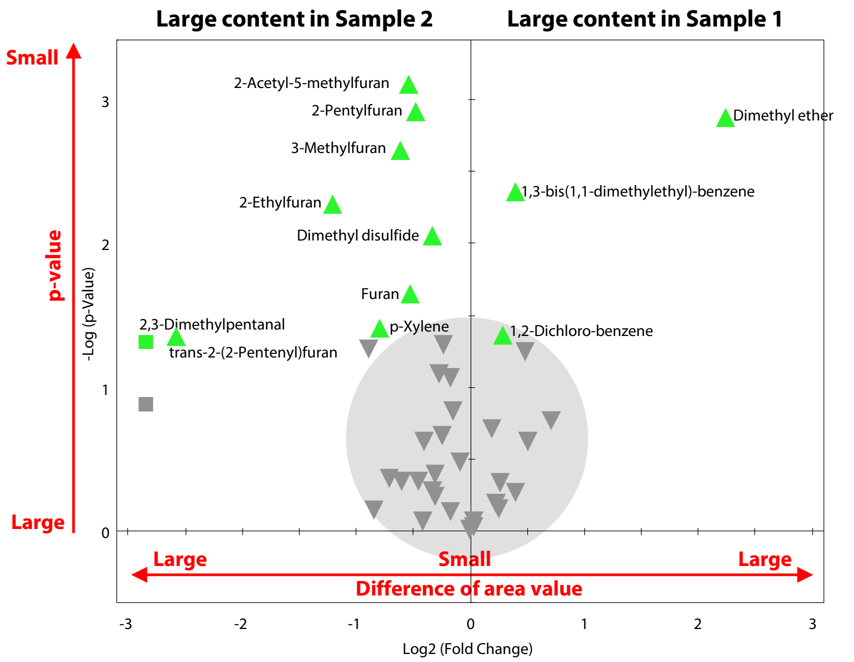
A two-group comparison using a volcano plot was carried out to investigate the differences between the samples in detail. The volcano plot technique makes it possible to discover components which have both large differences and statistically-significant differences by division of the average areas of the respective samples and a t-test.

First, Fig. 5 shows the results of a comparison of Sample 1 and Sample 2, which both had negative distributions in the first principal component of the PCA.

Here the green plots show components with p-values < 0.05, while those with gray plots are components with p-values > 0.05.

From p > 0.05, it was suggested that many components have no significant differences, such as the aldehydes and the terpenes.

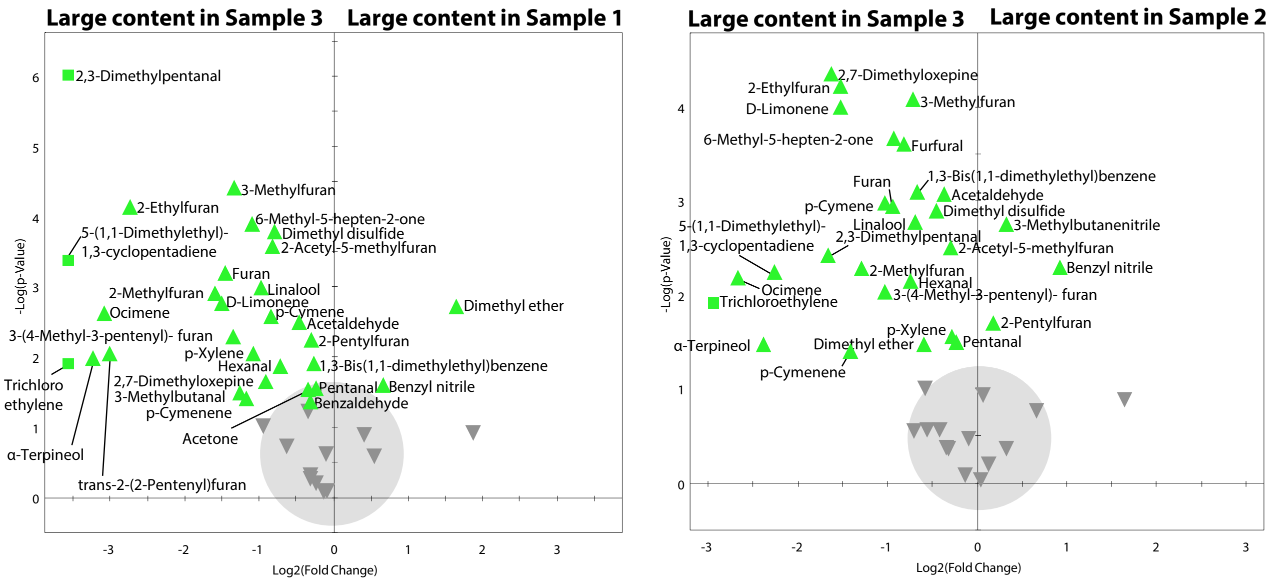
The components with p < 0.05 are furans, which have a sweet flavor. Although it was suggested that the contents of these components tend to be large in Sample 2, no remarkable difference was found.



Components with p > 0.05

1H-Pyrrole-2-carboxaldehyde
2-Butanone
2-Methylbutanal
2-Methylfuran
2,7-Dimethyloxepine
3-Furanmethanol
3-Methylbutanal
3-Methylbutanenitrile
3-Methylbutanoic acid
3-(4-Methyl-3-pentenyl)- furan
5-Methyl-2-furancarboxaldehyde
6-Methyl-5-hepten-2-one
Acetaldehyde
Acetone
Benzaldehyde
Benzeneacetaldehyde
Benzyl nitrile
Cymene
p-Cymenene
Dimethyl sulfide
Dimethyl trisulfide
Furfural
Hexanal
D-Limonene
Linalool
Pentanal

Fig. 5 Two-Group Comparison by Volcano Plot (Samples 1 and 2)
(Green; p < 0.05, Gray: p > 0.05, △, ▽: Contained in Both Samples, □: Contained in Only One Sample)



Components with p > 0.05

1H-Pyrrole-2-carboxaldehyde	Benzeneacetaldehyde
1,2-Dichloro-benzene	Dimethyl sulfide
2-Methylbutanal	Dimethyl trisulfide
2-Butanone	3-Methylbutanenitrile
3-Methylbutanoic acid	Furfural
3-Furanmethanol	
5-Methyl-2-furancarboxaldehyde	

Components with p > 0.05

1H-Pyrrole-2-carboxaldehyde	Benzeneacetaldehyde
1,2-Dichloro-benzene	Dimethyl sulfide
2-Methylbutanal	Dimethyl trisulfide
2-Butanone	3-Methylbutanal
3-Methylbutanoic acid	Acetone
3-Furanmethanol	Benzaldehyde
5-Methyl-2-furancarboxaldehyde	trans-2-(2-Pentenyl)furan

Fig. 6 Two-Group Comparisons by Volcano Plot
Left: Comparison of Sample 1 and Sample 3, Right: Comparison of Sample 2 and Sample 3
(Green: $p < 0.05$, Gray: $p > 0.05$, △, ▽: Contained in Both Samples, □: Contained in Only One Sample)

Fig. 6 shows the results of two-group comparisons of Sample 1 and Sample 3 (Left) and Sample 2 and Sample 3 (Right). As in the results of the principal component analysis, it was suggested that Sample 3 tends to have larger contents of many components than the other two samples. Among components with $p < 0.05$, focusing on those with large differences in area, the furans and monoterpenes, such as d-limonene, tended to be contained abundantly in Sample 3. The monoterpenes have a top-note flavor and are known to contribute to a fresh flavor with a strong impression.

■ Conclusion

The distinctive components between tomato juice products could be searched by capture of aromatic components with a headspace sampler and an non-targeted multivariate analysis by AnalyzerPro. An objective evaluation of the influence of differences in the types of raw materials/production process on aromatic components is possible by this approach.

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