SHIMADZU
Excellence in Science

Application News

No. C194

Liquid Chromatography Mass Spectrometry

# Food Metabolomics Analysis of Curry Using LC/MS/MS

In recent years, attention has been focused on the technology of metabolomics, which is defined as the comprehensive analysis of metabolites in vivo. Metabolomics is an academic field that comprehensively analyzes low-molecule metabolites such as amino acids and organic acids generated by the activities of cells to clarify differences among multiple sample groups. It is said that it permits exhaustive analysis more easily than other "omics" because the number of target components is small. Originally, it was a technique that has been developed with the expectation of results in the medical field, for example, in searches for diagnostic markers using clinical samples and etiological analyses using model animals. This analytical method is recently used more and more in the industrial and food fields to make comparisons among the products of different manufacturers, as well as to compare raw materials from different sources. The application of metabolomics not to living organisms but to food as covered here is called "food metabolomics".

This article covers an example of food metabolomics where we examined the changes observed when different ingredients are used in curries made in several households, and when a curry is left overnight, by comprehensively analyzing the hydrophilic components of the sauce using a high performance liquid chromatograph mass spectrometer (LC/MS/MS) and conducting verification through multivariate analysis.

T. Hattori, Y. Inohana

## Samples and Pretreatment

As samples, we prepared two home-made curries. One, made at household A, was designated Curry A, and the other made at household B was designated Curry B. Curry A left overnight was designated Curry A-2. Curry from a cafeteria was also sampled and was designated Curry C. Table 1 shows the details of the samples.

The samples were pretreated in accordance with the procedure in a metabolomics pretreatment handbook (Shimadzu catalogue No. C146-2181): 900 µL of a mixed solvent comprising water : methanol : chloroform in the ratio 1 : 2.5 : 1 was added to 50 µL of the curry sauce, and 630 µL of the supernatant after centrifugation was collected. 280 µL of ultra-pure water was added to this supernatant, further mixed, and 300 µL of the water/methanol layer obtained after centrifugation was subjected to ultrafiltration. Then 150 µL of it was sampled and dried to a solid state. The solid was dissolved in 150 µL of internal standard solution, then diluted tenfold with internal standard solution to generate the sample for LC/MS/MS analysis.

**Table 1 Sample Details**

| Sample | |
|---|---|
| Curry A | Homemade curry from household A |
| Curry A-2 | Curry A reheated after being kept in the refrigerator overnight |
| Curry B | Homemade curry from household B |
| Curry C | Curry from a cafeteria |

## Analysis Conditions

The LCMS™-8060 (Fig. 1) was used in conjunction with the ion-pair free LC/MS/MS method included in the LC/MS/MS Method Package for Primary Metabolites Ver. 2. This analytical method enables simultaneous analysis of 97 hydrophilic metabolites, such as amino acids, organic acids, nucleosides, and nucleotides, which are important in metabolome analysis in the life sciences field. Table 2 shows the analysis conditions for HPLC and MS.

**Table 2 Analysis Conditions**

| | |
|---|---|
| [HPLC conditions] (Nexera™ X2) | |
| Column | : Reversed-phase column |
| Mobile phases | : A) 0.1% Formic acid in water<br>B) 0.1% Formic acid in acetonitrile |
| Mode | : Gradient elution |
| Flow rate | : 0.25 mL/min |
| Injection volume | : 3 µL |
| [MS conditions] (LCMS-8060) | |
| Ionization | : ESI (Positive and negative mode) |
| Mode | : MRM |
| Nebulizing gas flow | : 3.0 L/min |
| Drying gas flow | : 10.0 L/min |
| Heating gas flow | : 10.0 L/min |
| DL temp. | : 250 °C |
| Block heater temp. | : 400 °C |
| Interface temp. | : 300 °C |

**Fig. 1 Nexera™ X2 and LCMS™-8060**

## Metabolome Analysis

Each curry sample was analyzed using LC/MS/MS, and the area ratio of each component with respect to the internal standard substance was determined using principal component analysis and the t-test provided by Traverse MS software. Fig. 2 shows a score plot and loading plot comparing Curry A and Curry A-2. There appears to be some difference between the groups in the score plot, but there is no clear distinction. Similarly, the t-test results for the comparison of Curry A and Curry A-2 are shown in Fig. 3. Although the components which are judged to be significantly different (p value < 5%) are limited, and even when these components are considered, no marked quantitative difference between the two samples could be confirmed. Based on these results, it is thought that there is not much elution or change of components within the ingredients in the sauce, as a result of keeping the curry overnight.

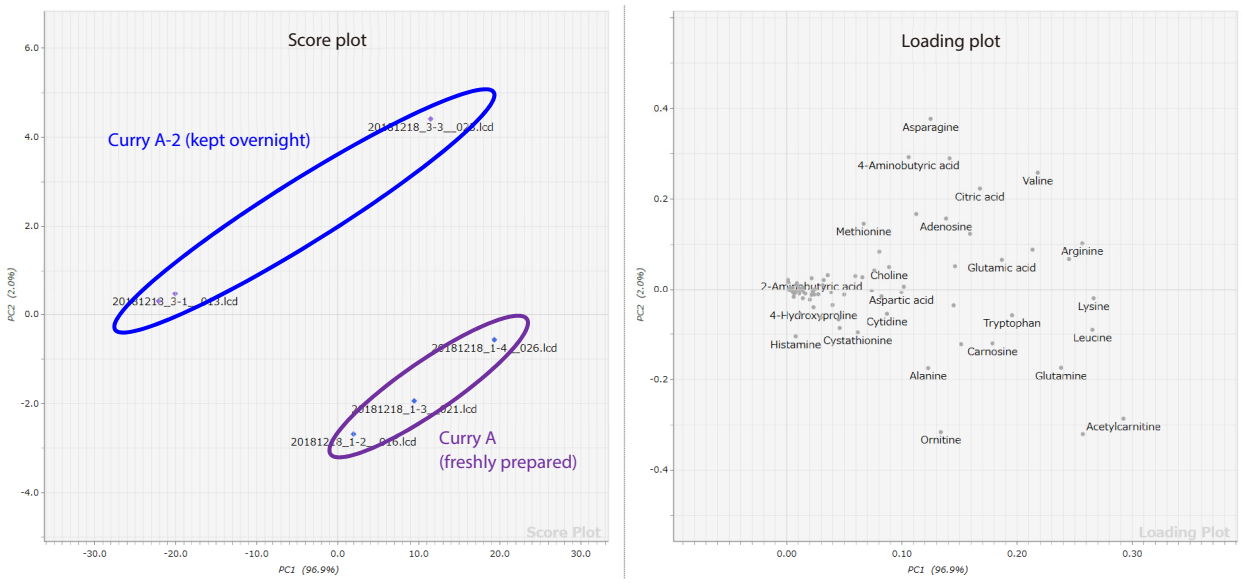


**Fig. 2 Principal Component Analysis Results for Curry A and Curry A-2**


**Fig. 3 t-test Results for Curry A and Curry A-2**

As indicated above, not much quantitative change in the components could be confirmed even after leaving the curry overnight. According to information from Internet searches, reasons cited for curry becoming tastier as a result of being left overnight are: elution of the "umami" components and sweetness components from the ingredients into the sauce, permeation of the sauce into the ingredients, mixing and blending of the flavors of the ingredients, spices and seasoning, and so on. It is generally said that the taste and flavor of stewed ingredients are transferred to the soup during cooking, and permeate from the soup to the ingredients while refrigerated. Considered on the basis of this information and the results of this analysis, even if curry is kept overnight, there is not much elution of "umami" components from the ingredients to the sauce, and we can suppose that the main reason that it is judged to be tastier may be that a variety of flavors and tastes are imparted to the ingredients, and "the ingredients and roux are blended" by repeated heating and cooling.

Next, we examined whether differences were observed among Curries A, B and C of different origins. The results of the t-tests and principal component analyses are shown in Figs. 4 and 5.

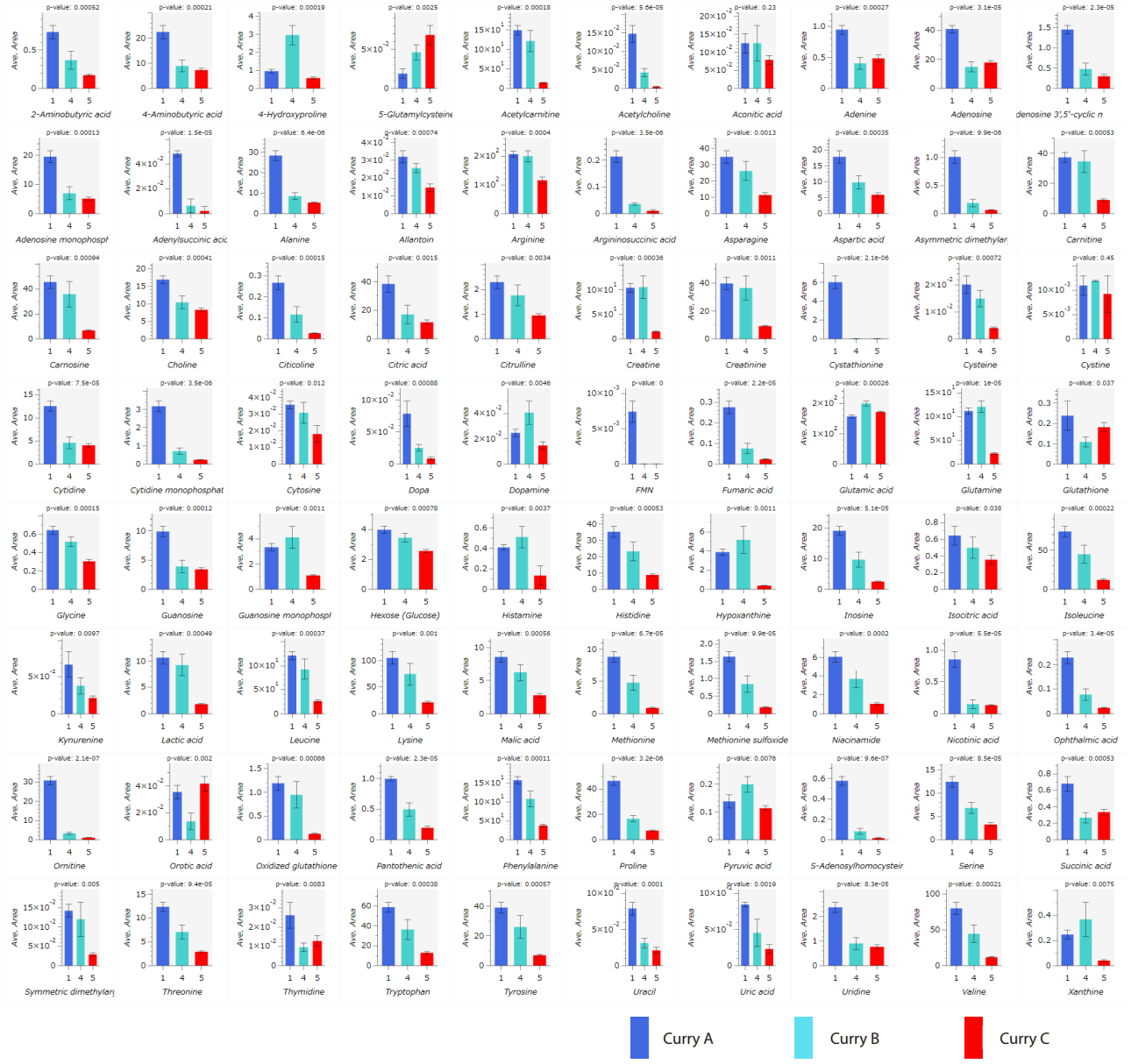


**Fig. 4 t-test Results for Curries A, B, and C**

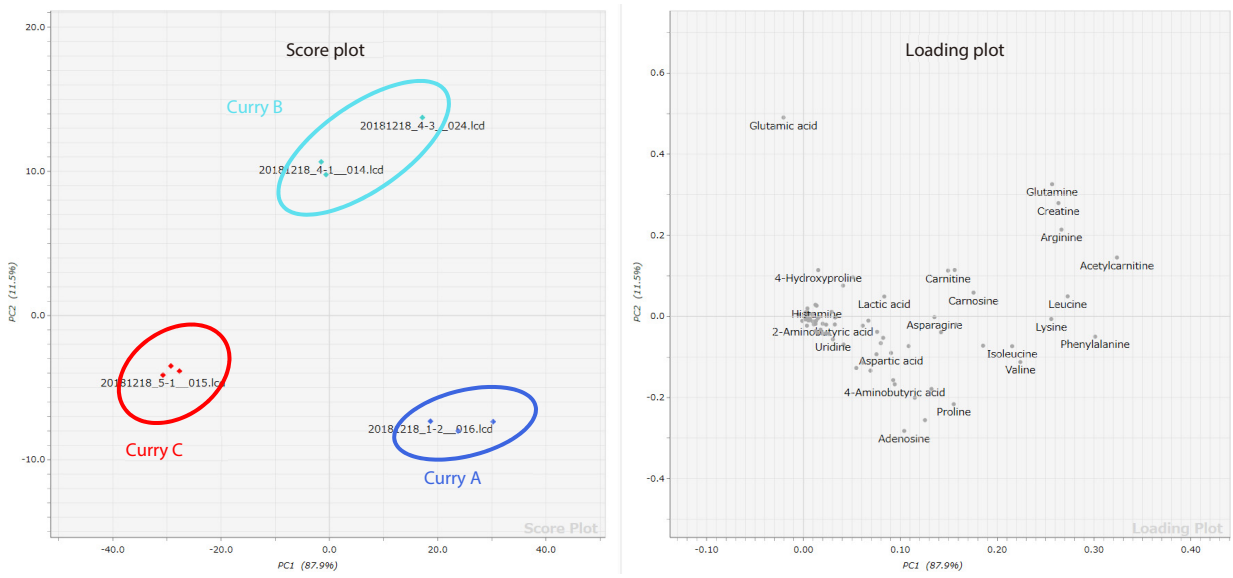


**Fig. 5 Principal Component Analysis Results for Curries A, B, and C**

On examining the results obtained, a tendency for the components contained to increase in the order: Curry A > B > C was observed. There seems to be a difference in the curry roux used, but another factor was that plenty of ingredients such as mushrooms and other vegetables were used in Curry A, so it is presumed that more components eluted into the sauce from these ingredients. In addition, it was found that Curry C tended to have a much smaller content of many of the components than the other two types of curry made in ordinary households. On the other hand, there was not much difference in the amount of glutamic acid (Fig. 6), which suggests that the taste could be adjusted by adding "umami" seasonings to insubstantial curries.

It is expected that the application of metabolomics to the food field in this way will enable a scientific approach to differences between samples, and combining it with sensory tests will lead to recognition as a more objective evaluation.

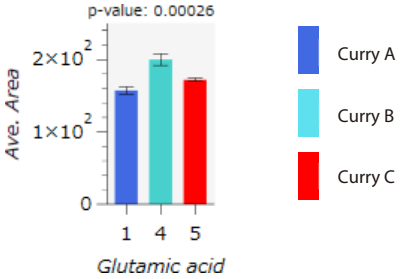


**Fig. 6 t-test Results for Curries A, B, and C (Extraction of Glutamic Acid)**



First Edition: Aug. 2019

Shimadzu Corporation
www.shimadzu.com/an/