SHIMADZU

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Widely targeted metabolomics of hydrophilic compounds using LC-MS/MS -How compounds change when curry is stored overnight?-

Takanari Hattori[1], Harumi Kubo[1], Yasuko Yamada[2], Jun Watanabe[1]

1 Shimadzu Corporation. 1, Nishinokyo-Kuwabaracho Nakagyo-ku, Kyoto 604–8511, Japan. 2 Shimadzu Techno-Research, Inc.,. 1, Nishinokyo-Shimoaicho, Nakagyo-ku, Kyoto, 604-8436 Japan

1. Overview

Widely targeted metabolomics using LC/MS/MS was applied to find out how compounds change when curry is stored overnight. The amounts of most compounds except for 6 metabolites were almost unchanged. It was suggested that compounds other than hydrophilic metabolites (e.g. spices and flavouring compounds, lipids and peptides) change and affect to the taste of curry that is stored overnight after cooking.

2. Introduction

Curry is a spicy cuisine originating in the Indian subcontinent that use a complex combination of spices or herbs. In Japan, curry powder came with the cooking method about 150 years ago from the United Kingdom and was combined with rice and spread as "rice curry". Meat, potatoes, carrots and onions are essential for Japanese curry. It is said that the taste become better when curry is stored overnight after cooking. Recently, increasing attention has been devoted to metabolomics to investigate the relationship between compounds and taste. In this study, widely targeted metabolomics using LC/MS/MS was applied to find out how compounds change when curry was stored overnight.

3. Materials and Methods

3-1. Sample.

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 stored overnight after cooking was designated Curry A-2. Curry from Shimadzu cafeteria was also sampled and was designated Curry C. Table 1 shows the details of the samples.

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

Figure 1 Curry C

3-2. Sample Pretreatment

The samples were pretreated in accordance with the procedure in a metabolomics pretreatment handbook (Shimadzu catalogue No. C146-2181): 900 µL mixed solvent (water : methanol : chloroform = 1 : 2.5 : 1) was added to 50 µL curry sauce and mixed by vortex mixer. 630 µL supernatant after centrifugation was collected and 280 µL ultra-pure water was added. After mixing vortex mixer and centrifugation, 300 µL water/methanol layer was collected and filtrated. 150 µL the filtrate was sampled and dried to a solid state. The solid was dissolved in 150 µL internal standard solution and diluted 10-fold with internal standard solution.

3-3. Analytical Conditions

The Nexera™ X2 and LCMS™-8060 (Figure 2) was used for metabolomics analysis of curries. The ion-pair free LC/MS/MS method included in the LC/MS/MS Method Package for Primary Metabolites Ver. 2. was used as analytical method. 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. Detailed analysis conditions of LC/MS/MS are shown below.

UHPLC (Nexera X2™ system)

Column: Discovery HS F5 (150 mmL. × 2.1 mmI.D., 3.0 µm)
Mobile phase A: 0.1% Formate/water
B: 0.1% Formate/acetonitrile
Flow rate: 0.25 mL/min
Injection vol.: 3 µL
Column temp.: 40°C

MS (LCMS-8060)

Ionization: ESI (Positive/Negative, MRM mode)
DL temp.: 250°C HB temp.: 400°C
Interface temp.: 400°C Nebulizing gas: 2.0 L/min
Drying gas: 10 L/min
Heating gas: 10 L/min

Figure 2 Nexera X2 + LCMS-8060

4. Result

Hydrophilic compounds in each curry sauce were analyzed using LC/MS/MS. Principal component analysis (PCA) and the t-test by Traverse MSTM software (Reifycs Inc., Japan) were conducted using the area ratio of each compound with internal standard substance. Figure 3 shows t-test results for the comparison of Curry A and Curry A-2. The amounts of most compounds were almost unchanged

. The amounts of 6 metabolites (adenylsuccinic acid, argininosuccinic acid, guanosine monophosphate, histamine, ornithine and oxidized glutathione) in Curry A sauce were obviously higher ($p < 0.05$) than those in Curry A-2 sauce. It was thought that there was not much elution from ingredients to sauce and change of hydrophilic compounds in sauce even if curry was stored overnight after cooking.

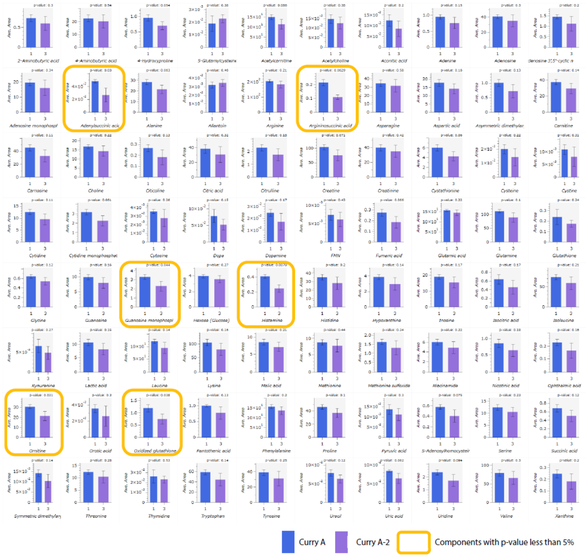

Figure 3 t-test Results for Curry A and Curry A-2

According to information from Internet searches, reasons why the taste of curry stored overnight after cooking become more delicious are: elution of the "umami“ compounds and sweetness compounds from the ingredients into the sauce, permeation of the sauce into the ingredients, mixing and blending of the flavors of the ingredients, spices and seasonings, and so on. It is generally said that the taste and flavor of stewed ingredients are transferred from ingredients to soup by heating and permeated from soup to ingredients by cooling. Considered on these information and the results of our metabolome analysis, even if curry is stored overnight after cooking, there is not much elution of "umami" components from ingredients to sauce. One of the reasons why the taste of curry stored overnight after cooking become more delicious is mixing and blending of compounds between ingredients and sauce by repeated heating and cooling.

Next, we examined the differences among Curries A, B and C. The results of PCA are shown in Figure 4.

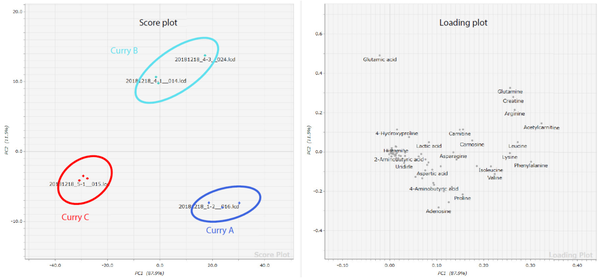

Figure 4 PCA Results for Curries A, B, and C

There was a tendency for amount of hydrophilic compounds contained in curry sauce to increase in the order Curry A > B > C. The different curry roux seems to be one of the factors, but plenty ingredients seem to be another factor. Mushrooms and other plenty vegetables were used in Curry A. So it is presumed that more compounds eluted into the sauce from these ingredients.

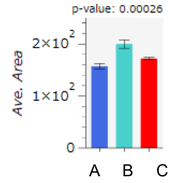

Figure 5 Glutamic Acid

The amount of most hydrophilic compounds in Curry C sauce was smaller than those in Curry A and B. However, there was not much difference in the amount of glutamic acid (Figure 5). So, It was suggested that the taste of Curry C with few ingredients could be adjusted by adding "umami" seasonings.

5. Conclusions

- Widely targeted metabolomics using LC/MS/MS was applied to find out how compounds change when curry is stored overnight.
- Not much change of hydrophilic compounds could be confirmed even if curry is stored overnight after cooking.
- It was suggested that one of the reasons why the taste of curry stored overnight after cooking become more delicious is mixing and blending of compounds between ingredients and sauce by repeated heating and cooling.