SHIMADZU APPLICATION NEWS

LA190-E014A

● HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No.L292A

Analysis of Amino Acids in Fermented Food and Drinks

Application News L284 introduced examples of analyzing sugars in fermented food products using HPLC. This Application News focuses on amino acids.

When analyzing amino acids with HPLC, it is important to select the optimal detection method to ensure high sensitivity and selectivity. In this regard, variations of pre- or post-column derivatization detection methods have been proposed, among which the post-column fluorescence derivatization method that uses o-phthalaldehyde (OPA) as the reaction reagent is particularly advantageous in sensitivity, selectivity, and operation simplicity. Thus this method is applied in a various fields, including the analysis of food products.

This Application News introduces examples of analyzing amino acids in fermented foods and drinks using the OPA derivatization method with the Shimadzu amino acid analysis system.

■ Simultaneous Analysis of 38 Amino Acids

The Shimadzu amino acid analysis system is available with two different separation methods, the Na type for analyzing amino acids hydrolyzed from protein and the Li type for analyzing free amino acids. This Application News introduces an example of analyzing 38 amino acids using the Li type system.

The amino acids are separated with a Li-type cation-exchange column, and secondary amines such as proline are converted to primary amines with hypochlorous acid aqueous solution. Then the primary amines are made react with the OPA reagent to be converted to fluorescent materials for detection. Table 1 shows the analytical conditions.

Fig. 1 shows the chromatogram for 38 amino acid standards. Each amino acid was added to purified water at a concentration of 5.0mmol/L (0.25mmol/L or 1.25mmol/L for some amino acids) and 10µL was injected. Table 2 shows the abbreviation for each amino acid.

Table 1 Analytical Conditions

Column	: Shim-pack Amino-Li (100mmL. × 6.0mmI.D.)
Mobile Phase	: Amino Acid Mobile Phase Kits (Li Type) Gradient Elution
Flow Rate	: 0.6 mL/min
Column Temp.	: 39˚C
Reagent	: Amino Acid Reagent Kits
Flow Rate of Reagent	: 0.3mL/min
Reaction Temp.	: 39˚C
Detection	: RF-10AXL Ex. at 350nm, Em. at 450nm

Table 2 Abbreviations of Amino Acids

Abbreviation	Amino Acid	Abbreviation	Amino Acid
P-SER	o-Phosphoserine	MET	L-Methionine
TAU	Taurine	ILE	L-Isoleucine
P-ET-AMINE	o-Phosphoethanolamine	CYSTATHIONINE	L-Cystathionine
ASP	L-Aspartic Acid	LEU	L-Leucine
OH-PRO	Hydroxy-L-proline	TYR	L-Tyrosine
THR	L-Threonine	PHE	L-Phenylalanine
SER	L-Serine	β-ALA	β-Alanine
ASN	L-Asparagine	β-A-I-B-A	DL-β-Aminoisobutyric Acid
GLU	Glutamic Acid	γ-A-B-A	γ-Aminobutyric Acid
GLN	L-Glutamine	TRP	L-Tryptophan
SAR	Sarcosine	HIS	L-Histidine
α-A-A-A	α-Aminoadipic Acid	3-ME-HIS	L-3-Methylhistidine
PRO	L-Proline	1-ME-HIS	L-1-Methylhistidine
GLY	Glycine	CARNOSINE	L-Carnosine
ALA	L-Alanine	ANSERINE	L-Anserine
CTRULINE	L-Citrulline	OH-LYS	Hydroxylysine
α-A-B-A	DL-α-Amino-n-butyric Acid	ORNITHINE	L-Ornithine
VAL	L-Valine	LYS	L-Lysine
CYS	L-Cystine	ARG	L-Arginine

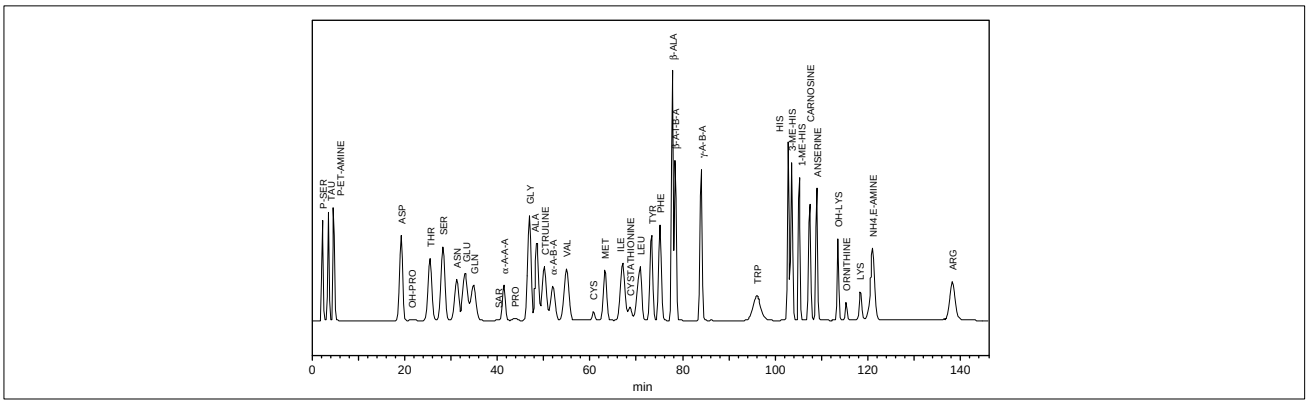

Fig.1 Chromatogram of 38 Standard Amino Acids Mixture

■ Analyzing Soy Sauce and Mirin (Sweet Cooking Sake)

The soy sauce was diluted by 200 times with a pH 2.2 citric acid (lithium) buffer. The mirin was diluted by 10 times, and then filtered through a membrane filter. The injection volume was 10μL for both samples (Fig. 2 and 3).

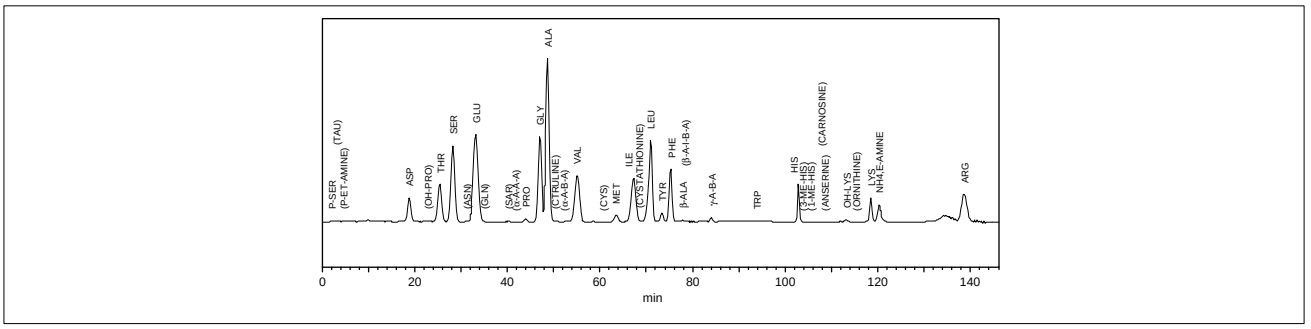

Fig.2 Analysis of Soy Sauce

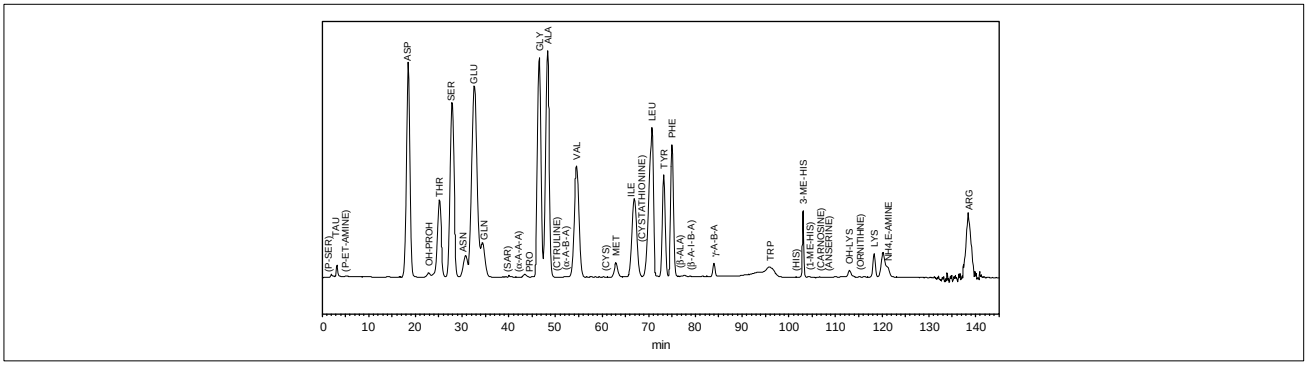

Fig.3 Analysis of Mirin

■ Analysis of Miso (Fermented Soybean Paste)

The miso sample was prepared in the procedure shown in Fig. 5, and 10μL was injected (Fig. 4).

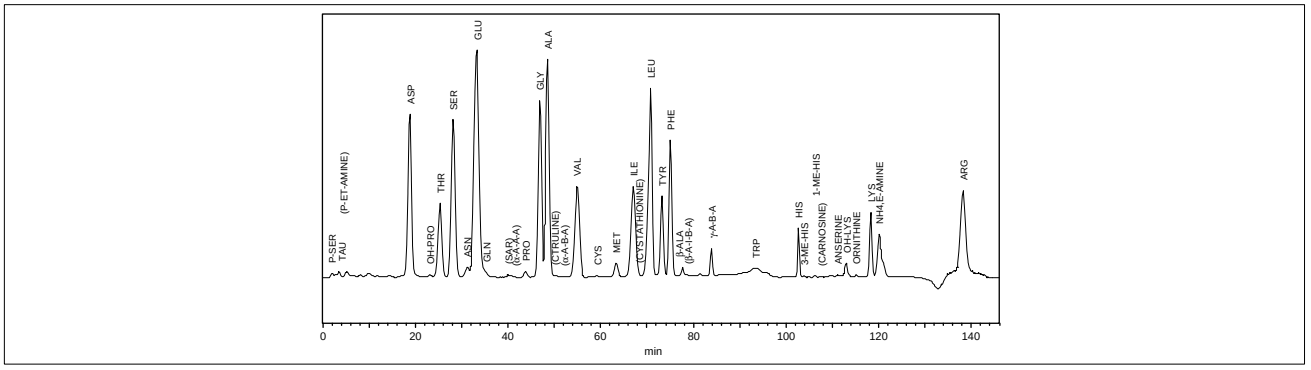

Fig.4 Analysis of Miso (Fermented Soybean Paste)

1000mg Miso in 5mL water
↓
Mixing
↓
Centrifuge 3000rpm×10min
↓
Supernatant
Filtration

↓
Ten times dilution with pH2.2 Lithium Citrate Buffer Solution
↓
Inject 10μL

Fig.5 Pretreatment of Miso (Fermented Soybean Paste)

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax. 81(3)3219-5710
Cable Add.:SHIMADZU TOKYO

Printed in Japan 3100-02406-10A-IK