



## Analysis of free amino acids in Grains (Physiological Fluid Analysis Method)

Grains are divided into staple grains, miscellaneous grains, pulses, and pseudocereals. Staple grains refer to the staple crops rice, wheat, and corn, while miscellaneous grains are a general term for the small caryopsis-bearing crops of the Gramineae family, such as barnyard millet, foxtail millet, and millet. Miscellaneous grains were once an important staple crop in Japan, but when rice production increased during the economic growth period, consumption and production of miscellaneous grains decreased. However, due to the growing health consciousness in recent years, the high nutritional value of miscellaneous grains has been reevaluated, and they are now used as a health food. Miscellaneous grains are also important staple crops, mainly in arid regions where it is difficult to grow rice and are mainly produced and consumed in large quantities in India and African countries.

In AS/AAA-040, we introduced the analysis of protein-constituting amino acids for nutritional evaluation of Grains. This report introduces an example of measuring free amino acids, which are involved in the taste and flavor, of staple grain (Non-glutinous rice) and three types of miscellaneous grains (Foxtail millet, Pilled millet, and Japanese barnyard millet) using LA8080 HIGH SPEED AMINO ACID ANALYZER (AminoSAAYA). The measurement results showed that miscellaneous grains had a higher amount of free amino acids than Non-glutinous rice, and that they all contained high amounts of Asparagine (AspNH<sub>2</sub>), GABA ( $\gamma$ -aminobutyric acid, g-ABA), and Arginine (Arg). Measuring free amino acids is useful for evaluating the characteristics of food.



**LA8080 HIGH SPEED  
AMINO ACID ANALYZER  
(AminoSAAYA)**

## Analytical Conditions, Sample Preparation

Table 1. Analytical conditions for Physiological fluid analysis method

Column	#2622PF 4.6 mm I.D. × 60 mm
Ammonia filter column	#2650L 4.6 mm I.D. × 40 mm
Guard column	#2619F 4.0 mm I.D. × 5 mm
Eluent	MCI BUFFER™ PF Kit (*)
Flow rate	0.35 mL/min
Column temperature	30 - 70 °C
Reaction reagent	Ninhydrin Coloring Solution Kit for HITACHI (*)
Reaction reagent flow rate	0.30 mL/min
Reaction temperature	135 °C
Detection wavelength	VIS 440 nm, 570 nm
Injection volume	20 $\mu$ L
Standard sample	Amino Acids Mixture Standard Solution, Type AN, B(High Range) (*) Asparagine(*), Glutamine(*), Tryptophan(*)

(\*) FUJIFILM Wako Pure Chemical Corporation

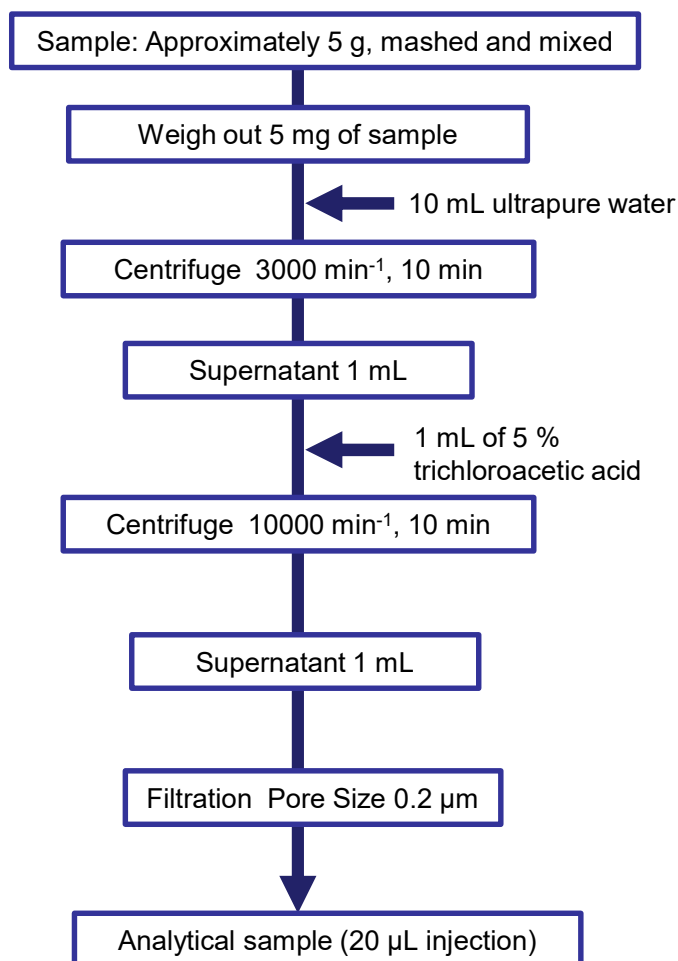


Fig.1 Preparation Method

## Amino Acid Analysis (Physiological Fluid Analysis Method)

- ✓ Miscellaneous grains contains more free amino acids than Non-glutinous rice.
- ✓ They all contained high amounts of Asparagine (AspNH<sub>2</sub>), GABA (γ-aminobutyric acid, g-ABA), and Arginine (Arg).

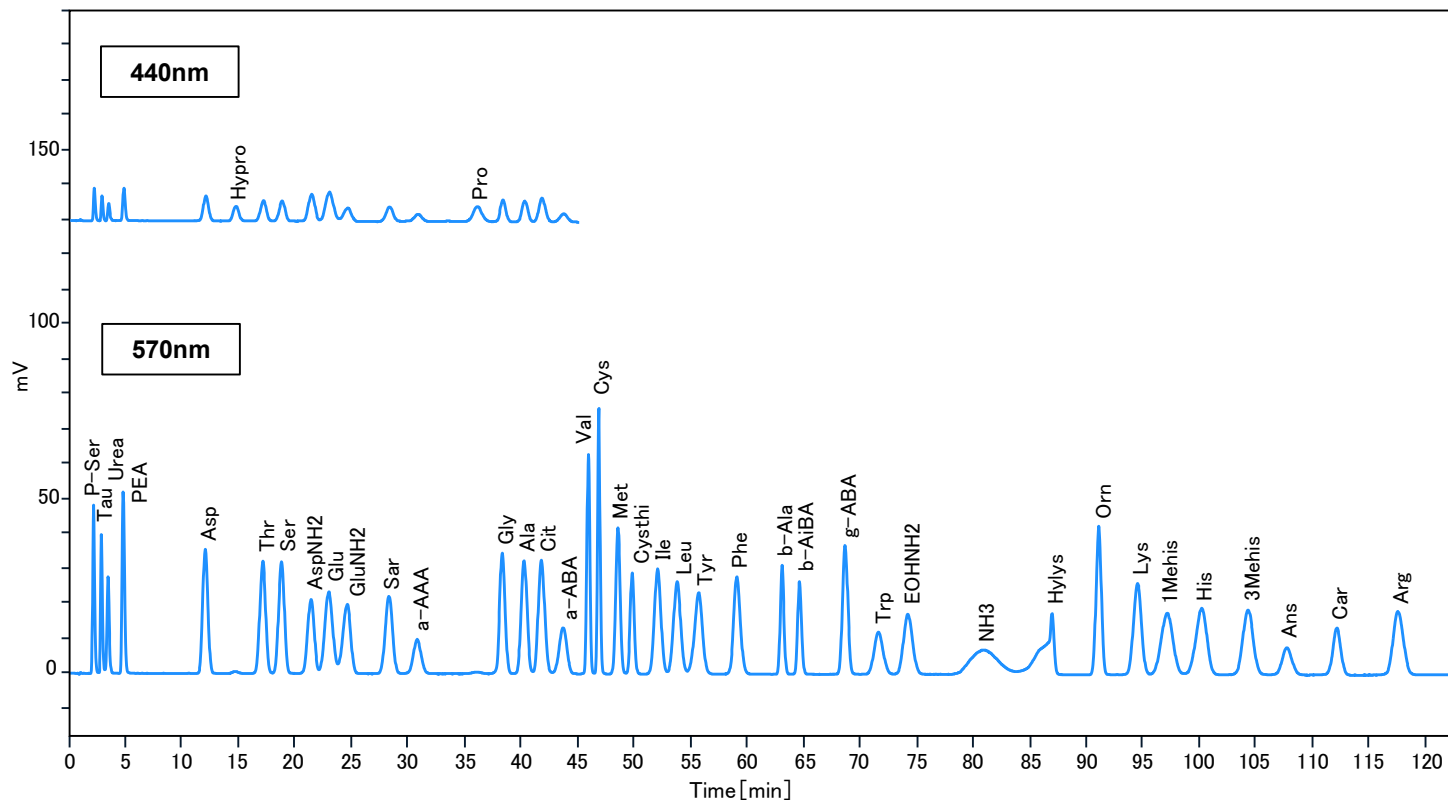


Fig.2 Analysis of Amino Acid Standard Solution (Physiological fluid analysis method)

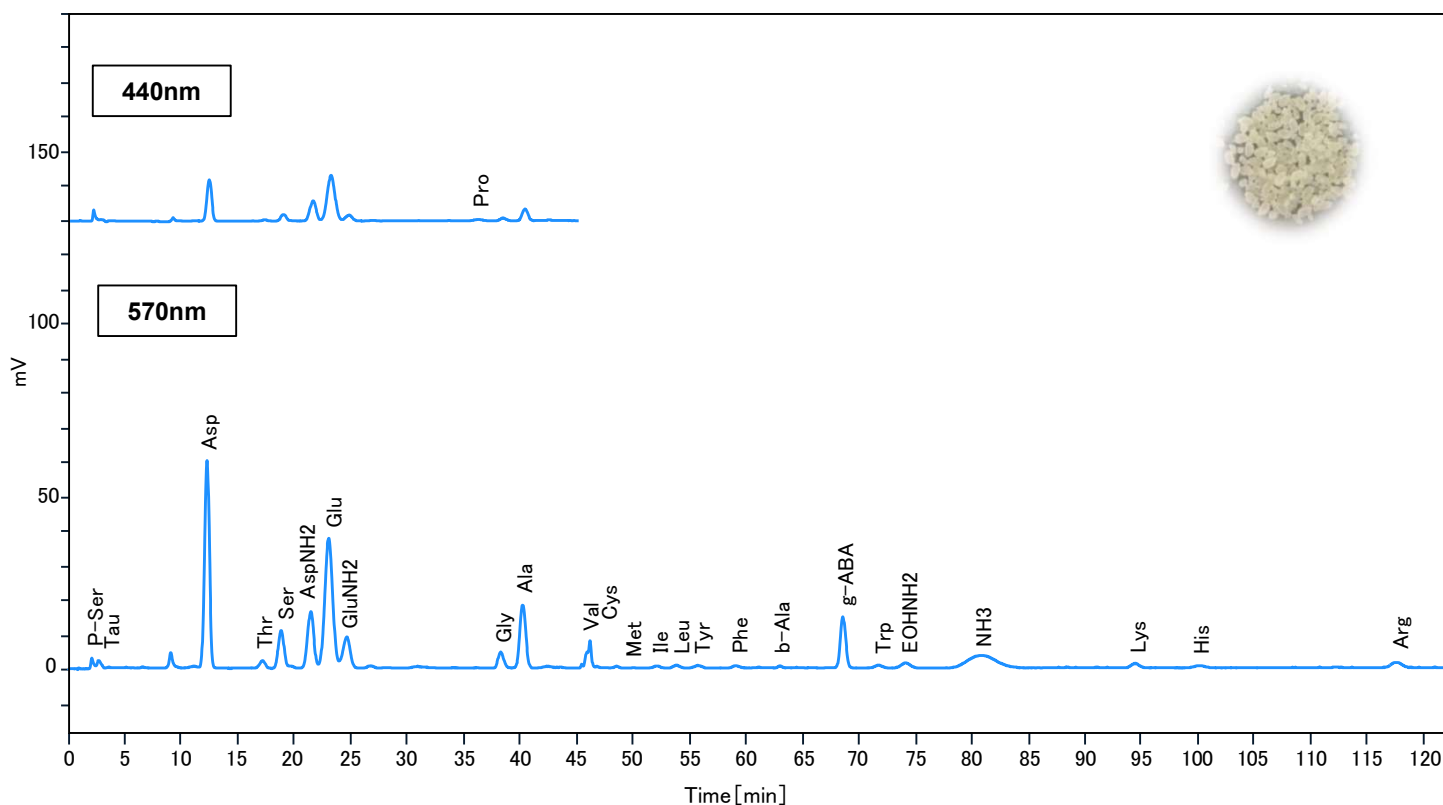


Fig.3 Analysis of Non-glutinous rice sample



## Amino Acid Analysis (Physiological Fluid Analysis Method)

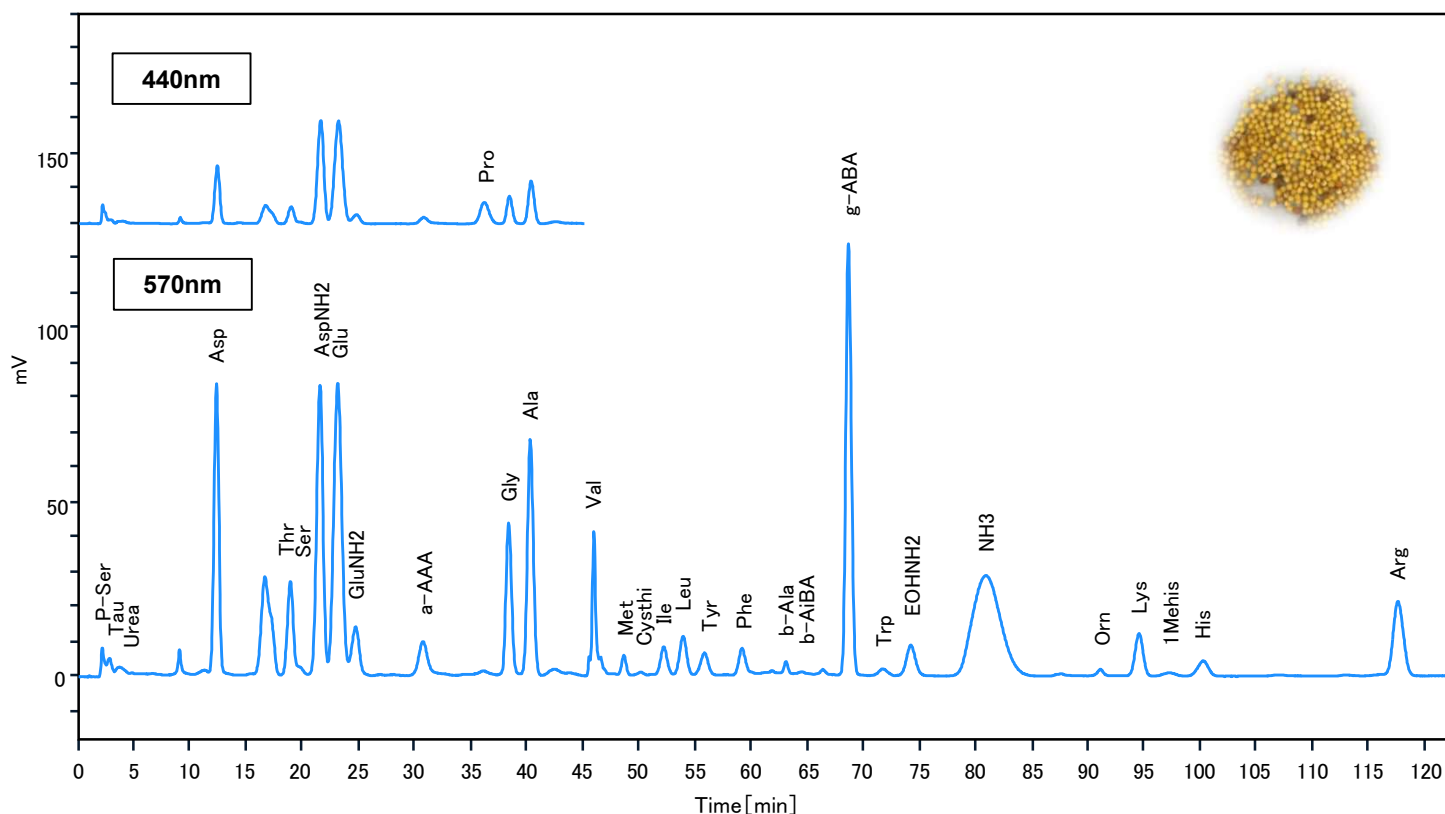


Fig.4 Analysis Foxtail millet sample

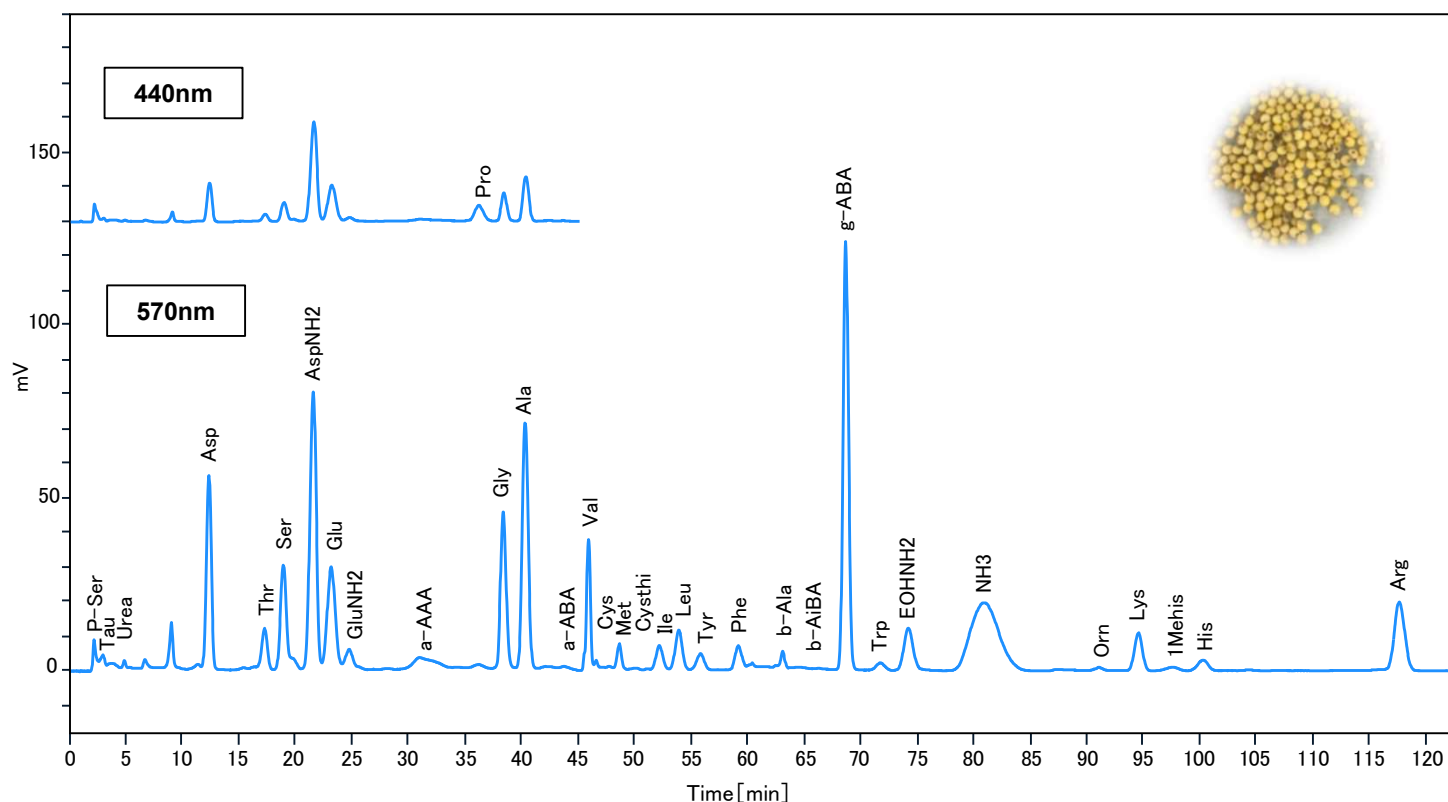


Fig.5 Analysis of Pilled millet sample

## Amino Acid Analysis (Physiological Fluid Analysis Method)

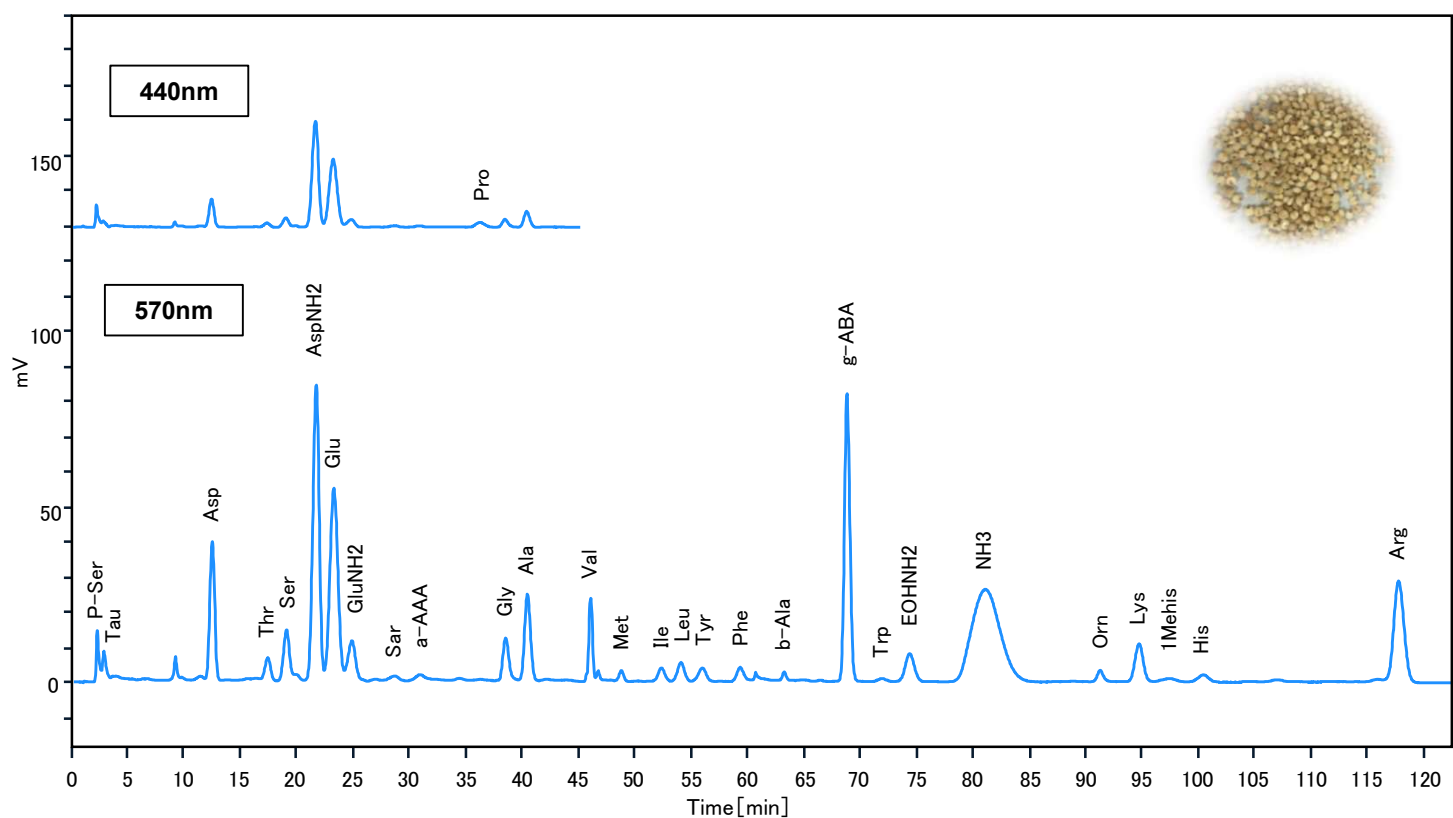


Fig.6 Analysis Japanese barnyard millet sample

## Comparison of amino acid content

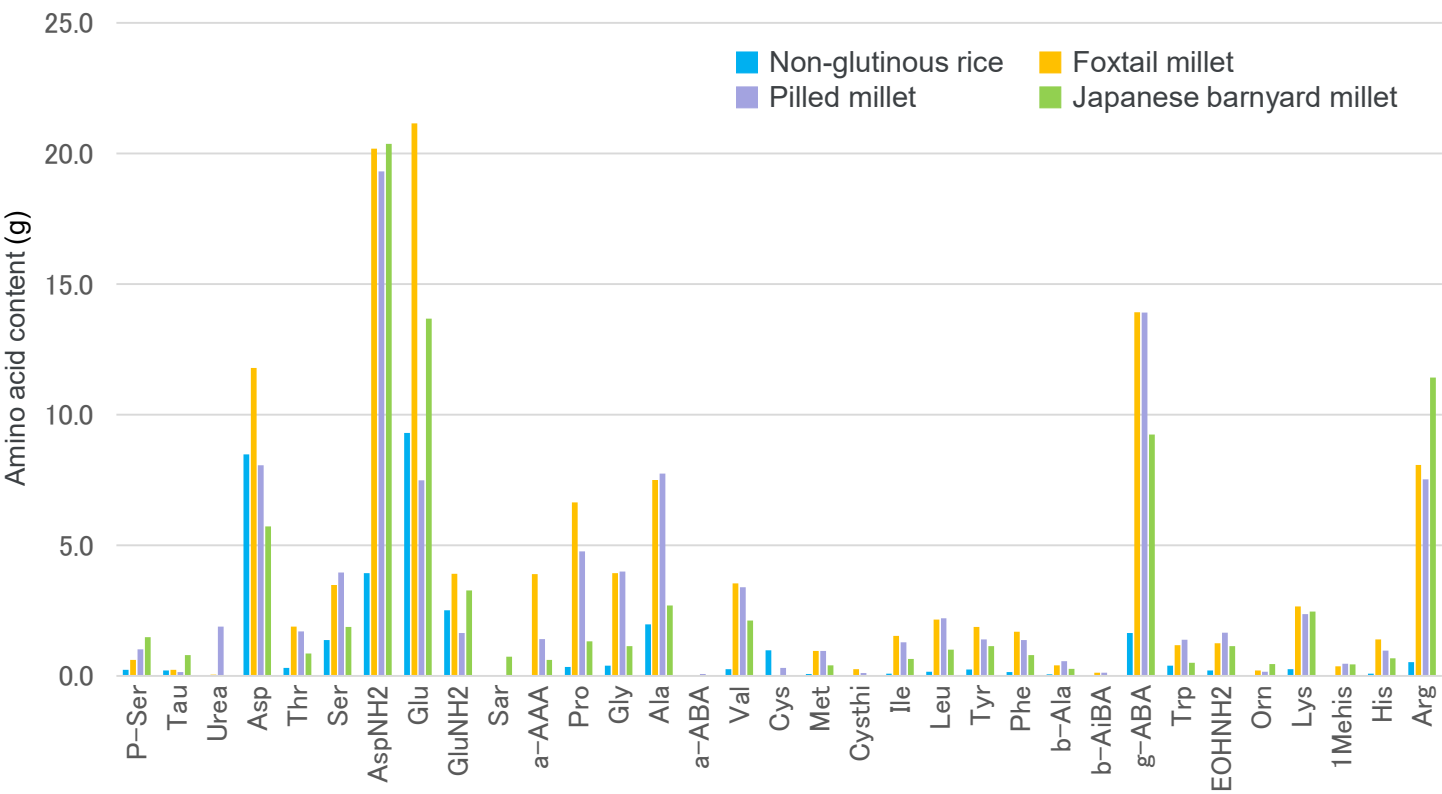


Fig.7 Free amino acids per 100g sample (g)



## List of Amino Acids

Abbrev.	Amino acid	Molecular weight	Std. concentration (nmol/ 20 $\mu$ L)
P-Ser	Phosphoserine	185.1	1
Tau	Taurine	125.2	1
PEA	Phosphoethanolamine	141.1	1
Urea	Urea	60.1	40
Asp	Aspartic acid	133.1	2
Hypro	Hydroxy proline	131.1	2
Thr	Threonine	119.1	2
Ser	Serine	105.1	2
AspNH <sub>2</sub>	Asparagine	132.1	2
Glu	Glutamic acid	147.1	2
GluNH <sub>2</sub>	Glutamine	146.2	2
$\alpha$ -AAA	$\alpha$ -Amino adipic acid	161.2	1
Sar	Sarcosine	89.1	5
Pro	Proline	115.1	2
Gly	Glycine	75.1	2
Ala	Alanine	89.1	2
Cit	Citrulline	175.2	2
$\alpha$ -ABA	$\alpha$ -Amino-n-butyric acid	103.1	1
Val	Valine	117.1	2
Cys	Cystine	240.3	2
Met	Methionine	149.2	2
Cysthi	Cystathionine	222.3	1
Ile	Isoleucine	131.2	2
Leu	Leucine	131.2	2
Tyr	Tyrosine	181.2	2
Phe	Phenylalanine	165.2	2
$\beta$ -Ala	$\beta$ -Alanine	89.1	2
$\beta$ -AiBA	$\beta$ -Amino iso butyric acid	103.1	2
$\gamma$ -ABA	$\gamma$ -Amino-n-butyric acid	103.1	2
Trp	Tryptophan	204.1	2
EOHNH <sub>2</sub>	Ethanol amine	61.1	2
NH <sub>3</sub>	Ammonia	17.0	2
Hyls	Hydroxylysine	162.2	2
Orn	Ornithine	132.2	2
Lys	Lysine	146.2	2
1Mehis	1-Methylhistidine	169.2	2
His	Histidine	155.2	2
3Mehis	3-Methylhistidine	169.2	2
Ans	Anserine	240.3	2
Car	Carnosine	226.2	2
Arg	Arginine	174.2	2

These data are an example of measurement; the individual vales can not be guaranteed.

Specifications in this catalog are subject to change with or without notice, as Hitachi High-Tech Analysis Corporation continues to develop the latest technologies and products for our customers.