

## Amino Acid Contents in the Hydrolysates of Fulvic Acids Extracted from Decomposing Plant Residues

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### 腐熟植物遺體에서 抽出한 Fulv 酸 加水分解 溶液中의 Amino 酸含量

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#### SUMMARY

Sixteen amino acids in the hydrolysates of fulvic acid fraction from 7 plant materials were determined.

Analyzed amino acids were aspartic acid, glutamic acid, arginine, histidine, lysine, glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, serine, threonine, proline, and methionine. Four crop residues, wild grass cuttings and forest tree litters were put under investigation.

1. The content of amino acids in fulvic acid fractions extracted after 90 days of compositing ranged from 0.15% to 0.53% by dry weight. The highest value was found in the fulvic acids of wild grass cuttings and the lowest in those of wheat straw, being equivalent to 1/5-1/31 of those found in humic acids.
2. The group of neutral amino acids shared the largest portion followed by acidic and basic amino acids.
3. Arginine was not detected in fulvic acid fractions from well decomposed residues.
4. Aromatic amino acids, phenylalanine and tyrosine, were virtually absent in fulvic acid fractions.
5. Glycine, glutamic acid and aspartic acid were the 3 major amino acids contained in fulvic acids of well decomposed residues. With glutamic acid and aspartic acid excluded, the decreasing order of concentration of amino acids was roughly in parallel with the increasing order of molecular weight.

#### INTRODUCTION

It has been well known that the fulvic acid fractions have lower molecular-weight, lower carbon, lower nitrogen but higher oxygen contents than humic acids (1, 2, 11). Fulvic acids contain more acidic functional groups. The low molecular weights and high acidities of fulvic acids make them more soluble than humic

acids, and they are known to exhibit special functions as regards pedogenic processes, pesticide transformations, and chelation with metals (1, 9, 11). The characteristic role of fulvic acids in the formation of Spodosols under coniferous forest canopy has drawn much attention (9).

There are authors who do not recognize structural difference between fulvic and humic acids except in

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molecular weight (3, 12). It has been further suggested that fulvic acids are made up of phenolic and benzenecarboxylic acids joined by hydrogen-bonding to form polymeric structures of considerable stability (10). But evidences obtained from IR spectrum analyses clearly show structural differences between fulvic and humic acids. Results given from chemical analyses for oxygen-containing functional groups such as carboxyl, phenolic, alcoholic, carbonyl, and quinonic groups, explicitly demonstrate the differences between structural units of fulvic and humic acids (4, 5). Fulvic acids are predominantly aliphatic. On the contrary humic acids possess strong aromatic characteristics.

Among the structural units of fulvic and humic acids there are amino acids which constitute presumably the greatest portion of nitrogen-containing structural units. It is expected that qualitative and quantitative comparisons of amino acid contents of fulvic and humic acids may yield some results which would be used as evidences to show structural differences. Experimental results from this study for fulvic acids will be compared with those obtained for humic acids already reported in previous papers (4, 5, 6, 7, 8).

## MATERIALS AND METHOD

### 1. Preparation of fulvic acids

Fulvic acids were extracted from decomposing residues of 7 plant materials: straws of rice, barley, wheat, and rye, wild grass cuttings, and litters of coniferous and deciduous forest trees, respectively. Before the extraction of fulvic acids they were put under decomposition for specified periods of 45 and 90 days.

### 2. Hydrolysis of fulvic acids

Hydrolysis of fulvic acids was effected with 10 ml of 6N-HCl added to 0.5g of powdered dry sample in a hydrolysis container. It was then stoppered under nitrogen atmosphere and allowed to stand for 24 hrs at 105°C. The hydrolyzed solution was filtered through Toyo No. 6 filter paper and concentrated by using a Vapormixer at a temperature below 40°C. The filtrate was adjusted to pH 2.2.

### 3. Analysis of amino acids

An LKB 4150  $\alpha$ -Amino Acid Analyzer was employed for the quantification of 16 amino acids: aspartic acid, glutamic acid, arginine, histidine, lysine, glycine, alanine, valine, leucine, isoleucine, phenylal-

anine, tyrosine, serine, threonine, proline, and methionine.

## RESULTS AND DISCUSSION

The concentration of amino acids in fulvic acid fractions varies greatly not only from sample to sample but among stages of decomposition as shown in Table 1. At the end of 90 days of humification there was 45.74  $\mu$ mol of amino acids in the fulvic acid fraction of wild grass cuttings, which was the highest value observed. The fulvic acid fraction from wheat straw contained the lowest value of 13.39  $\mu$ mol. These are equivalent to 0.53% and 0.15% of fulvic acid by weight, respectively. Proportions of amino acids in fulvic acid fractions were 0.34%, 0.17%, 0.15%, 0.21%, 0.53%, 0.30%, and 0.29% for rice straw, barley straw, wheat straw, rye straw, wild grass cuttings, deciduous litter, and coniferous litter, respectively. These are about 1/5 to 1/31 of amino acids contained in humic acids extracted from the same plant materials (6, 7, 8). This agrees well with the widely accepted fact that the nitrogen content of fulvic acids is less than that of humic acids, because amino acids are the major nitrogenous component of fulvic and humic acids (1, 2, 11, 12). There was erratic changes in the relative concentration of amino acids occurred during humification (Fig. 1). In general neutral amino acids were in the largest concentrations followed by acidic and basic amino acids (Table 1), as were also reported for humic acids (6, 7, 8). With the progression of humification arginine seemed to disappear and was completely absent in the end of 90 days of decomposition.

It is very interesting to observe the analytical result that very little or virtually none of phenylalanine and tyrosine was detected in fulvic acid fractions. Phenylalanine and tyrosine are aromatic amino acids, and the absence of them in fulvic acids should be presented as a strong evidence of extremely weak aromaticity of fulvic acids, which confirms experimental results obtained from spectrometric and chemical analyses (5).

Glycine, glutamic acid and aspartic acid belong to the group of amino acids which were found in the highest concentration in fulvic acids extracted from well decomposed plant materials as well as in those from raw plant residues. Incidentally the order of decreasing concentration of amino acids is roughly in parallel with the increasing order of molecular weight when glutamic and aspartic acids are excluded from

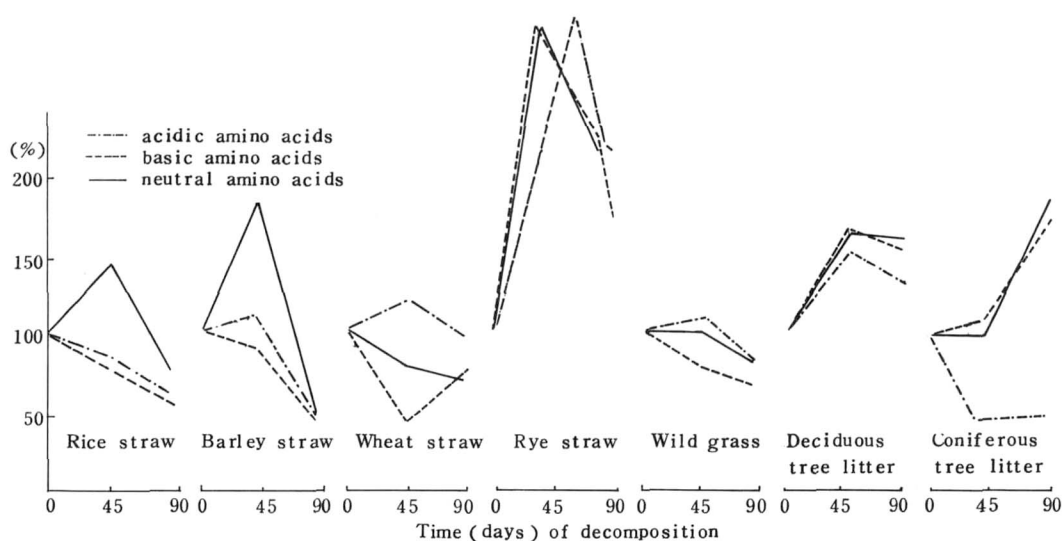


Fig. 1. Percent molar changes of amino acids in the hydrolysates of fulvic acids extracted from decomposing plant residues.

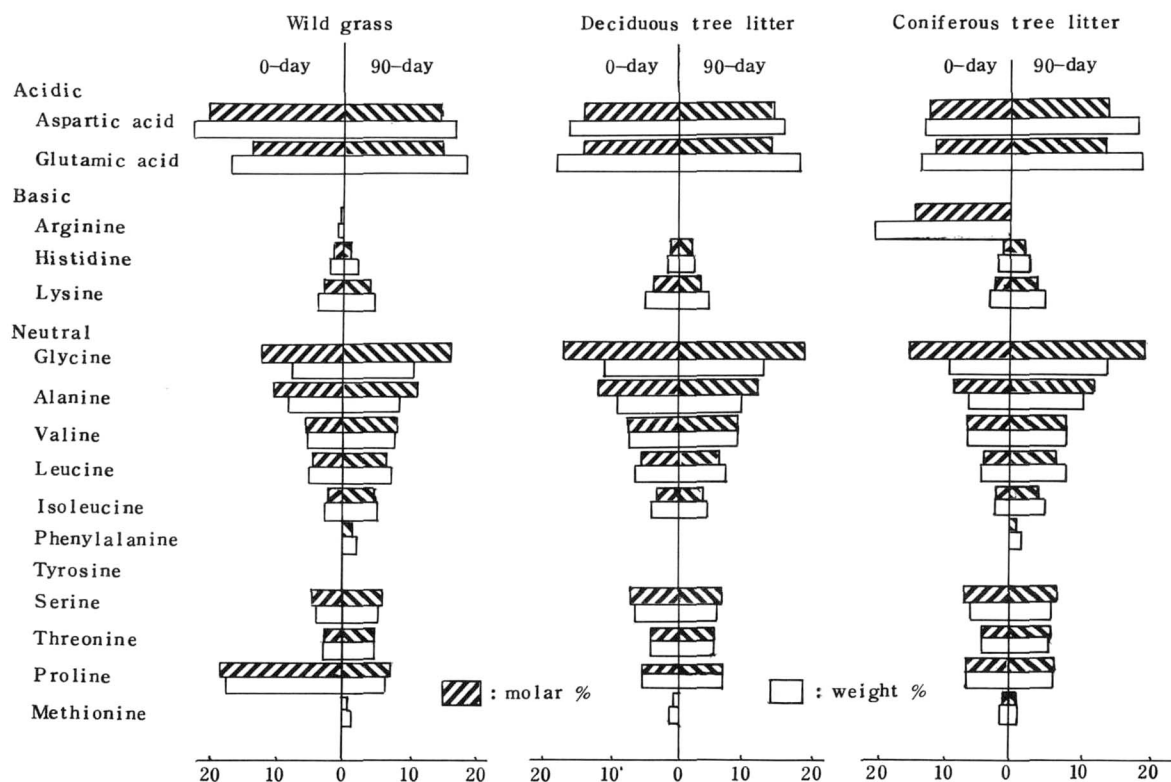


Fig. 2. Percent molar and weight distribution of amino acids in the hydrolysates.

**Table 1. Concentrations of amino acids in the hydrolysates of fulvic acids extracted from decomposing plant residues**

Amino acids	Concentration, $\mu\text{mol/g}$ dry matter																				
	Rice straw			Barley straw			Wheat straw			Rye straw			Wild grass			Deciduous litter			Coniferous litter		
	0-d	45-d	90-d	0-d	45-d	90-d	0-d	45-d	90-d	0-d	45-d	90-d	0-d	45-d	90-d	0-d	45-d	90-d	0-d	45-d	90-d
Acidic																					
Aspartic acid	6.39	5.46	4.35	4.17	3.79	1.93	2.27	2.00	1.64	1.26	3.86	2.04	12.27	7.76	6.77	2.38	3.75	3.72	2.11	2.52	3.80
Glutamic acid	7.78	6.45	4.20	6.31	5.59	2.42	2.88	0.19	1.99	1.95	7.96	3.46	8.32	8.46	6.75	2.36	4.28	3.65	1.98	2.21	3.67
Subtotal acidic	14.17	11.91	8.55	10.48	9.38	4.35	5.15	2.19	3.63	3.21	11.82	5.50	20.59	16.22	13.52	4.74	8.03	7.37	4.09	4.73	7.47
Basic																					
Arginine	1.11	-	-	-	-	-	-	-	-	-	-	-	0.10	0.08	-	-	-	-	2.54	-	-
Histidine	0.59	0.63	0.25	0.46	0.49	0.16	0.23	0.22	0.19	0.18	0.27	0.53	0.93	0.87	0.61	0.25	0.44	0.34	0.20	0.47	0.38
Lysine	1.55	1.91	1.50	1.43	1.62	0.73	0.60	0.76	0.59	0.45	1.26	0.81	1.89	2.23	1.73	0.67	0.99	0.90	0.45	0.74	0.87
Subtotal basic	3.25	2.54	1.75	1.89	2.11	0.89	0.83	0.98	0.78	0.63	1.53	1.34	2.92	3.18	2.34	0.92	1.43	1.24	3.19	1.21	1.25
Neutral																					
Glycine	6.36	6.54	5.03	4.53	5.39	2.11	2.83	3.24	2.45	1.86	4.58	2.37	7.60	8.77	7.49	2.88	5.03	5.06	2.67	3.00	5.37
Alanine	4.48	14.40	3.56	3.87	10.84	1.57	2.07	1.74	1.11	1.34	4.09	2.08	6.46	7.26	5.16	2.05	3.19	3.26	1.57	1.45	3.31
Valine	3.29	3.35	2.11	2.10	2.21	1.04	1.46	1.27	0.81	1.10	2.63	1.28	3.36	4.37	3.58	1.31	1.78	2.11	1.15	1.28	2.17
Leucine	2.73	2.88	1.71	1.69	2.08	0.74	0.95	0.64	0.49	0.53	2.07	0.82	2.73	3.92	2.98	1.02	1.63	1.63	0.73	0.91	1.70
Isoleucine	1.15	1.53	1.26	0.72	1.28	0.47	0.36	0.48	0.34	0.28	1.39	0.57	1.31	2.32	1.89	0.63	0.85	0.89	0.39	0.59	1.02
Phenylalanine	-	0.18	-	-	4.16	-	0.37	-	-	-	0.23	-	-	0.70	0.63	0.07	0.26	-	-	-	0.22
Tyrosine	-	-	-	-	-	-	-	-	-	-	0.16	0.25	-	0.28	-	-	-	-	-	-	-
Serine	2.34	2.17	1.22	1.40	1.47	0.66	1.15	0.83	0.91	0.54	1.75	0.78	2.86	3.31	2.54	1.26	2.28	1.74	1.27	1.13	1.74
Threonine	1.21	1.47	0.88	0.97	0.99	0.49	0.58	0.53	0.70	0.40	0.95	0.47	1.92	2.30	2.21	0.75	1.41	1.40	0.77	0.75	1.40
Proline	3.33	3.17	2.44	2.03	2.55	1.07	2.64	1.02	1.64	-	3.96	1.71	11.20	3.85	3.09	0.96	1.56	1.80	1.20	0.90	1.75
Methionine	0.10	0.09	0.31	0.11	1.23	-	0.18	0.05	0.14	0.11	0.30	0.15	0.15	1.26	0.31	0.14	0.30	-	0.17	0.04	0.14
Subtotal neutral	24.99	35.78	18.52	17.42	32.20	8.15	12.59	9.80	8.59	6.16	22.11	10.48	37.59	38.34	29.88	11.07	18.29	17.91	9.92	10.05	18.82
Total	42.41	50.23	28.82	29.79	43.69	13.39	18.57	12.97	13.00	10.00	35.46	17.32	61.10	57.74	45.74	16.73	27.75	26.52	17.20	15.99	27.54

comparison: glycine M.W. 75.07, alanine M.W. 89.09, serine M.W. 105.09, proline M.W. 115.13, valine M.W. 117.15, threonine M.W. 119.12, leucine M.W. 131.17, isoleucine M.W. 131.17, (aspartic acid M.W. 133.10), lysine M.W. 146.19, (glutamic acid M.W. 147.13), methionine M.W. 149.21, histidine M.W. 155.16, phenylalanine M.W. 165.19, arginine M.W. 174.20, and tyrosine M.W. 181.19.

There was very little difference between deciduous and coniferous forest litters concerning the content and concentration of amino acids after 90 days of humification. On the other hand a drastic variation was measured among crop residues of rice, barley, wheat and rye straws (Table 1).

Results from this study make it difficult to support the suggestion that essentially there is no structural difference between humic acids and fulvic acids, humic acids being mere polymers of fulvic acids (3, 12). Absence of aromatic amino acids and molar distributions of other amino acids in fulvic acid fractions lead to the conclusion that there are many distinctive dissimilarities between fulvic and humic acids in all respects of not only physicochemical but also purely chemical characteristics.

## 摘 要

벗짚, 보릿짚, 밀짚, 호밀짚의 4가지 作物遺體와 山野草,闊葉樹와 針葉樹 落葉等 7가지 植物遺體를 腐熟시키면서 經時的으로 試料를 採取하여 抽出한 fulvic acid를 加水分解시킨후, amino acid를 定量分析한 結果 aspartic acid, glutamic acid, arginine, histidine, lysine, glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, serine, threonine, proline, methionine 등 16種의 amino acid가 檢出되었다.

1. 腐熟 90日後에 抽出한 fulvic acid중에는 0.15% 내지 0.53%의 amino acid가 함유되어 있었고, 山野草에 가장 많이 그리고 밀짚에 가장 적게 함유되어 있었다. Humic acid의 함량에 비해 1/5 내지 1/31의 정도로 함량이 적었다.

2. Neutral amino acids, acidic amino acids, basic amino acids의 順으로 많이 함유되어 있었다.

3. 腐熟化가 잘된 試料에서는 arginine이 검출되지 않았다.

4. Aromatic amino acid인 phenylalanine과 tyrosine은 일반적으로 검출되지 않았다.

5. Glycine, glutamic acid와 aspartic acid가 주요 amino acid였으며 glutamic acid와 aspartic acid를 제외하고 비교해본 결과, 각 amino acid에서 분자량이 낮을수록 함유된 농도가 높은 경향이였다.

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