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# Bio-available amino acids extraction from soil by demineralized water and 0.5 M ammonium acetate

*Short Communication*

**P. Formánek**[1], **B. Klejdus**[2], and **V. Vranová**[1]

[1] Department of Geology and Pedology, Mendel University of Agriculture and Forestry in Brno, Brno, Czech Republic
[2] Department of Chemistry and Biochemistry, Mendel University of Agriculture and Forestry in Brno, Brno, Czech Republic

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**Summary.** The extraction and comparison of soil bio-available amino acids using either demineralised water (DEMI $H_2O$) or 0.5 M ammonium acetate (0.5 M AAc) solution is reported. Results show that the extraction by 0.5 M AAc is a better method to assess the concentration of bio-available amino acids in soil than DEMI $H_2O$ due to higher extraction efficiency and better amino acid protection against microbial degradation during processing.

**Keywords:** Soil – Amino acids – Extraction

## Introduction

It has long been known that soil amino acids in their ''free state'' can be uptaken either by mycorrhizal or nonmycorrhizal plant roots (Read, 1991; Chapin et al., 1993) and their role in nitrogen nutrition of plants is of intensive interest. Such bio-available amino acids are present either in solution as dissolved matter or as particulate matter loosely bound to organic or inorganic residues. However, they represent only very small fraction of the total soil amino acid pool (Kielland, 1994; Lipson et al., 2001). Thus, it is desirable to develop a robust extraction procedure applicable to the release of amino acids from soil exchangeable sites, especially in the case of positively charged, tightly bound amino acids which are poorly extracted by water (Schmidt et al., 1960; Paul and Schmidt, 1961) despite being readily uptaken by plants. In addition, any method developed must avoid or minimise protein hydrolysis which may give mis-leading results.

To date, demineralized water (DEMI $H_2O$) and 0.5 M ammonium acetate (0.5 M AAc) are considered to be the best extractants for this purpose. A water to soil ratio of at least 3:1 (v/w) is used frequently (Kielland, 1995; Nordin et al., 2001; Lipson and Monson, 1998) because it mimicks water flow through soil pore spaces. However, conflicting results have been reported. For example, Abuarghub and Read (1988) report neglible protein hydrolysis using 0.5 M AAc to extract free amino acids from soil due to its neutral pH (6.8), whereas, Schmidt et al. (1960) report a weak hydrolytic effect of 0.5 M AAc whilst attempting to recover tryptophan from soil. Furthermore, Jones (1999) has reported that the half-life of water extracted amino acids is relatively short, even when maintained at 5°C, because water does not provide any protection of amino acids against microbial degradation, and the extraction procedure takes several hours. Thus, DEMI $H_2O$ extracts give underestimated level of the actual bio-available amino acid present in soil.

To the best of our knowledge, there are no literature reports of a comparative study to assess extraction capability of DEMI $H_2O$ *versus* 0.5 M AAc for naturally occuring bio-available amino acids from soil because the former is regarded as a weak amino acid extractant. To this effect, we report a series of experiments which compare soil amino acids extraction by DEMI $H_2O$ and 0.5 M AAc, as both, amino acids concentrations and protein hydrolysis were taken into account.

## Material and methods

*Soil samples preparation*

Ten random sub-samples were taken from Ah horizon of mountain meadow on March 13, 2004. Their physical and chemical properties are as

follows: clay 19.2, silt 26.6, sand 54.2 (%), pH ($H_2O$) 4.3; pH (10 mM $CaCl_2$) 3.8; $C_{tot}$ 3.17%; $N_{tot}$ 0.31%, $NH_4^+$ 11.80 $\mu g \cdot g^{-1}$ dry soil, $NO_3^-$ 7.41 $\mu g \cdot g^{-1}$ dry soil. Sub-samples were immediately transported to the laboratory, sieved through <5 mesh size and stored for 12 h at 4°C in plastic bags until extraction procedures were started. Every sub-sample was prepared separately. No mixing to form composite samples were used.

*Soil extraction*

The extraction of amino acids from soil depends on ratio between soil and extractant. Hence, in both cases, 50 g of individual soil sub-samples was extracted, in parallel, with either DEMI $H_2O$ (200 ml) or 0.5 M AAc (200 ml) contained in 500 ml polyethylene bottles. After 1 h shaking, soil suspensions were filtered through paper filters and glass fiber GF 30 filters (55 mm, 1 $\mu$m, Schleicher & Schuell, Germany) at 6°C. Freezed extracts (18°C) were reduced to 20 times their original concentration by lyophylization. The lyophylized extracts were dissolved in 0.1 M HCl (500 $\mu$l) and filtered through a nylon membrane filter (13 mm, 0.45 $\mu$m, Chromatography Research Supplies, Adison USA).

*Amino acids measurement by HPLC*

The measurement of 17 amino acid concentrations were performed using an HP 1100 liquid chromatograph (Hewlett Packard, Wilmington, DE, USA) with fluorometric detector FLD HP 1100 operating at 450 nm (Ex = 340 nm). L-Cystine was taken into account due to its relatively high amount in bacterial spores, especially the coat layers. Separation was carried out with a Zorbax Exlipse AAA Rapid Resolution (4.6 × 150 mm, 3.5 $\mu$m paticle sizes, Agilent Technologies, USA). A linear gradient profile of mobile phase, consisting of 40 mM $Na_2HPO_4$, pH 7.8 (solvent A) and ACN/MeOH/water 45:45:10 (v/v) (solvent B), 0% B (0–1.9 min), 0–57% (1.9–18.1 min), 57–100% (18.1–18.8 min), 100% (18.8–22.3 min), 100–0% (22.3–23.2 min) and 0% (23.2–26 min) was applied at a flow rate of 2.0 ml $min^{-1}$. The column was equilibrated for 5 min under initial conditions prior to injection of the next samples. The column temperature was 40°C. For determination of amino acids from soils extracts precolumn derivatization with *o*-phthalaldehyde (OPA) was used.

*Measurement of proteolytic effect of 0.5 M AAc*

The hydrolytic effect of 0.5 M AAc was verified on the basis of casein-hydrolysis measurement. Sodium caseinate was dissolved either in demineralized water or 0.5 M AAc to a final concentration of 1%. The resultant solutions were stored in plastic bottles (n = 3 for each of the extractants) for 7 days at 4°C. Thereafter, the modified procedure described in work of Nannipieri et al. (1979) for soil casein-protease activity measurement was used starting as follows: 2 ml of stored 1% casein solution were mixed with 2 ml 0.05 M tris-HCl bufer (pH 8.5) and 1 ml of 17.5% trichloroacetic acid, immediately filtered and 1 ml of filtrate mixed with 3.7% aqueous $Na_2CO_3$, and 1 ml 0.06% aqueous $CuSO_4$. After mixing and 30 min incubation at room temperature, 1 ml of Folin-Ciocalteau reagent (diluted 1:3 water) was applied and after 5 min incubation at 37°C and 20 min at room temperature, casein hydrolysis was determined at 578 nm. The calibration lines were prepared from stock solution of L-tyrosine in DEMI $H_2O$ and 0.5 M AAc.

*Statistical analysis*

Statistical comparisons of both extraction procedures were performed by One-Way Anova (Statistica 6.0) for every amino acid separately (n = 10).

## Results and discussion

The results of the experiment are summarized in Table 1. The concentration of individual amino acids in DEMI $H_2O$ extracts relate quite well to monthly mean concentrations of amino acids in organic soil of tundra as reported by Kielland (1995) even though it is specific to soil and plant coverage. The total bio-available amino acids nitrogen amounted to 0.43 (DEMI $H_2O$) and 1.29 (0.5 M AAc) $\mu g\ g^{-1}$ in dry soil and both are similar to concentration ranges reported by other researchers (Lipson et al., 1999; Abuarghub and Read, 1988). From results obtained, it is evident that 0.5 M AAc

**Table 1.** Concentrations of individual amino acids in DEMI $H_2O$ and 0.5 M AAc extracts ($ng \cdot g^{-1}$ dry soil). Mean ± SD (n = 10)

| L-Amino acid | 0.5 M AAc extraction | Water extraction | W/AAc (%) |
|---|---|---|---|
| Asparate | 775.58 ± 695.11 | 106.45 ± 203.12 | 13.74 |
| Glutamic acid | 1911.15 ± 1547.98 | 307.15 ± 632.09 | 16.07 |
| Serine | 269.85 ± 159.39 | 187.63 ± 94.55 | 69.53 |
| Histidine | 41.69 ± 64.67 | 30.51 ± 56.71 | 73.17 |
| Glycine | 1521.35 ± 799.00 | 397.43 ± 382.85 | 26.12 |
| Threonine | 282.44 ± 893.16 | 5.04 ± 10.58 | 1.78 |
| Arginine | 642.56 ± 656.25 | 172.10 ± 160.05 | 26.78 |
| Alanine | 525.28 ± 421.61 | 107.12 ± 188.04 | 20.39 |
| Tyrosine | 158.83 ± 135.97 | 132.65 ± 118.82 | 83.51 |
| Cystine | 775.38 ± 502.40 | 294.66 ± 257.98 | 30.79 |
| Valine | 193.88 ± 145.66 | 59.69 ± 96.79 | 30.79 |
| Methionine | 196.46 ± 428.48 | 20.66 ± 65.32 | 10.51 |
| Phenylalanine | 251.08 ± 168.92 | 284.11 ± 420.26 | 113.16 |
| Isoleucine | 117.61 ± 83.87 | 31.47 ± 69.74 | 26.75 |
| Leucine | 342.27 ± 216.77 | 166.14 ± 232.16 | 48.54 |
| Lysine | 225.70 ± 707.19 | 58.09 ± 178.80 | 25.73 |
| Proline | 906.68 ± 452.41 | 849.02 ± 188.82 | 90.33 |

**Table 2.** Statistically significant differences in extraction efficiencies for individual amino acids. Comparisons were performed by One-Way ANOVA (n = 10). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

| L-Amino acids | P-values |
|---|---|
| Asparagine | $0.009^{**}$ |
| Glutamic acid | $0.0071^{**}$ |
| Serine | 0.178 |
| Histidine | 0.686 |
| Glutamine | $0.001^{***}$ |
| Threonine | 0.339 |
| Arginine | $0.041^{*}$ |
| Alanine | $0.01^{**}$ |
| Tyrosine | 0.652 |
| Cysine | $0.015^{*}$ |
| Valine | $0.026^{*}$ |
| Methionine | 0.216 |
| Phenylalanine | 0.820 |
| Isoleucine | $0.022^{*}$ |
| Leucine | 0.097 |
| Lysine | 0.477 |
| Proline | 0.579 |

gives much better extraction yield with highest relative differences in the cases of threonine, methionine and asparate. From all of the 17 amino acids detected in our samples only phenylalanine was more efficiently extracted by DEMI $H_2O$. Statistically significant differences between both tested extractants are for individual amino acids given in Table 2.

Hydrolytic effect of 0.5 M AAc on sodium caseinate was not present, as no differences from dissolution of casein sodium in demineralized water were detected. In our opinion, the extraction by 0.5 M AAc is a better method to assess the concentration of bio-available amino acids in soil than DEMI $H_20$ due to higher extraction efficiency and better amino acid protection against microbial degradation during processing.

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**Authors' address:** Dr. Pavel Formánek, Department of Geology and Pedology, Mendel University of Agriculture and Forestry in Brno, Zemedelska 3, 613 00 Brno, Czech Republic,
Fax: +420 5 45 21 14 22, E-mail: formanek@mendelu.cz