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The effect of extractants on degradation of L-glutamate and L-arginine in the course of shaking and filtration at low temperature

Short Communication

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Summary. The effects of demineralized water (DEMI H_2O) and 0.5 M ammonium acetate (0.5 M AAc) on losses of L-glutamic acid and L-arginine in the course of shaking and filtration at low temperature (6 °C) were tested. The concentration of L-glutamic acid decreased by 6.3% in DEMI H_2O and by 4.9% in 0.5 M AAc, whereas the L-arginine concentration decreased by 6.0% (DEMI H_2O) and 10.7% (0.5 M AAc). We found a significantly ($P < 0.05$) higher degradation of L-arginine in 0.5 M AAc compared with that of DEMI H_2O.

Keywords: Demineralized water – Ammonium acetate – Amino acids – Losses – Extraction

Abbreviations: DEMI H_2O, dermineralized water; 0.5 M AAc, 0.5 M ammonium acetate; OPA, *o*-phthalaldehyde

Introduction

Bio-available amino acids in soil are of intensive interest due to their role in the nitrogen nutrition of plants. It has been reported by many authors that mycorrhizal and non-mycorrhizal plants can uptake amino acids directly without previous mineralization (Read, 1991; Chapin et al., 1993; Raab et al., 1996). The role of amino acids in plant nutrition is very important, especially in ecosystems where unfavourable climatic and edaphic conditions lower nitrogen mineralization. Such conditions are typical in arctic, boreal and alpine ecosystems (Rehder and Shafer, 1978; Fisk and Schmidt, 1995; Kaye and Hart, 1997). Amino acids available for direct plant and microbial uptake are present in soil solutions with concentrations ranging from low μM to several mM, dependent on soil type and proximity to the rhizosphere (Jones, 1999).

The half-life of soil amino acids in ''free state'' is very short and depends on many factors, such as temperature, concentration, soil type, soil depth and properties of particular horizons (water content, sorption, microbial community structure, release of extracellular enzymes, etc.) (Holden, 1962; Jones, 1999). Different approaches have been used to extract bio-available amino acids from soils; the best of them seems to be extraction by cold demineralized water (DEMI H_2O) or 0.5 M ammonium acetate (0.5 M AAc). So far, the extraction procedure using DEMI H_2O is preferred in most studies as it simulates amino acids in soil pore water (Kielland, 1995; Nordin et al., 2001; Lipson and Monson, 1998). Ammonium acetate (0.5 M) extraction was used by Abuarghub and Read (1988) due to its better extraction efficiency and a reduced chance of hydrolytic cleavage.

Apart from extraction efficiency, it would also be valuable to know which of the two extractants gives better protection to amino acids that have been extracted from soils, since the mean half-life of bio-available amino acids in top soils is 2.9 h even at 5 °C (Jones, 1999). The extraction of bio-available amino acids is performed in several steps at low temperatures (4–6 °C) and takes several hours, including 1 h shaking of soil with extractant, and filtration, which takes several hours or can be performed overnight. Frozen extracts (−18 °C) are then reduced by lyophilization.

With regard to the short half-life of the amino acids at low temperatures and a relatively long extraction procedure,

we attempted to simulate the extraction experiment and replenish missing knowledge about amino acid losses in the two extractants (DEMI H_2O and 0.5 M AAc). For this purpose, we took demineralized water and 0.5 M ammonium acetate solutions of two L-amino acids (glutamic acid and arginine) which were detected in their highest concentrations in the mineral soil of alpine meadows studied in Moravian-Silesian Beskids (the northeast part of the Czech Republic).

Materials and methods

Simulation of extraction of soil amino acids

Two amino acids (L-glutamic acid and L-arginine), each in the amount of 100 μM, were dissolved in one liter of DEMI H_2O and 0.5 M AAc (stock solutions). A 10-ml aliquot (control) was removed immediately from each solution and frozen at −18 °C for subsequent lyophilization. The concentration of control samples measured after lyophilization was taken as a line (100%) according to which the effect of shaking and filtration on both amino acid concentrations was consequently evaluated. The effect of lyophilization was not evaluated in this study. Nevertheless, the concentrations of both amino acids measured in control samples were finally lower than 100 μM. In five repetitions, 50-ml aliquots from each of the stock solutions were shaken in polyethylene bottles for 1 h at 6 °C, then filtered through paper filters at the same temperature. Frozen extracts (−18 °C) were reduced to 20 times their original concentration by lyophilization. The lyophilized extracts were dissolved in 0.1 M HCl (500 μl) and filtered through a nylon membrane filter (13 mm, 0.45 μm, Chromatography Research Supplies, Adison, USA).

Measurement of amino acids by HPLC

Both amino acid concentrations were measured using an HP 1100 liquid chromatograph (Hewlett Packard, Wilmington, DE, USA) with fluorometric detector FLD HP 1100 operating at 450 nm (Ex = 340 nm). Separation was carried out with a Zorbax Exlipse AAA Rapid Resolution (4.6 × 150 mm, 3.5 μm particle sizes, Agilent Technologies, USA). A linear gradient profile of the 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 (Formánek et al., 2005).

Statistical analysis

Statistical comparisons of both decreases in amino acid concentrations in DEMI H_2O and 0.5 M AAc were performed by One-Way Anova (Statistica 6.0) for $n = 5$.

Results and discussion

The results obtained in this study demonstrated that the concentration of L-glutamic acid being dissolved in DEMI H_2O decreased in the course of shaking and filtration at low temperature by $6.3 \pm 0.60\%$ (mean ± 1SE) with respect to the control sample (100%), whilst arginine decreased by $6.0 \pm 0.50\%$. When 1 h shaking and filtration was performed with 0.5 M ammonium acetate solution, the concentrations of glutamic acid and arginine decreased by $4.9 \pm 0.85\%$ and $10.7 \pm 1.4\%$, respectively, when related to the control sample. No significant effect of extractant on the loss of glutamic acid in the course of shaking and the filtration procedure ($P > 0.05$) was detected. However, arginine loss was significantly ($P < 0.05$) higher, thus indicating a less protective effect of 0.5 M ammonium acetate. The results obtained in this study with two amino acids commonly present in soil of the studied alpine meadow show that the degree of degradation of amino acids in the course of shaking and filtration depends on both type of extractant and individual amino acids. Just as Jones et al. (2002) hypothesize higher protection of amino acids against microbial degradation in 2 M KCl compared with water, we found unexpectedly higher losses of arginine in 0.5 M ammonium acetate when compared with demineralized water.

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