Quantitative determination of amino acids in potatoes A-11.4

Key words

Instrumental HPTLC - quantitative TLC analysis - densitometry (fluorescence) - prechromatographic derivatization (dansylation) - food analysis - quality control

Scope

Quantitative analysis or "fingerprinting" of amino acids in feed stuff is required for evaluating quality, stability, and occasionally the provenience of the product. This concerns free as well as protein bound amino acids, after hydrolysis.

The method described is for the determination of free amino acids in potatoes; it can be readily adapted for other tasks.

Determination of free amino acids in potatoes is required, e.g. when by-products of potato processing plants are to be used for animal feed, for which the content of free amino acids is regulated. Nowadays, pure amino acids are obtained from potato juice.

Potato juice is deproteinated by precipitation with ethanol. The free amino acids are derivatized with dansyl chloride, chromatographed on silica gel and scanned by fluorescence. The limits of determination are in the low nanogram range.

Advantages of performing this analysis by instrumental TLC

- Easy sample preparation
- High detection sensitivity
- Suitable for routine analysis with large sample throughput

Reagents

Ethanol
dist. water
Acetone
n-Butanol
Diethylether
Dansylchloride, 75 mg in 30 mL acetone
Sodium bicarbonate, 1 % in water
Sodium hydroxide 1 n
Ethylendiaminetetraacetate-Disodium salt (Titriplex III)
Paraffin - n-hexane 2:5

Sample preparation

- Add 10 mL ethanol to 3 mL potato juice at room temperature.
- Filter the precipitated protein.
- Evaporate the light brown solution to dryness at 40°C.
- Dissolve residue in 3 mL water.

Dansylation:

- Add 0.5 mL dansylchloride solution to 1 mL of the conditioned potato juice and adjust to pH 8 with the prepared sodium bicarbonate solution.
- For the derivatization-reaction the solution is placed in the dark (at room temperature) for 16 hours.
- Transfer the reaction-solution with acetone - water 7:3 in a 10 mL measuring flask and fill up.
- For the determination of arginine this solution is diluted 1:10 and can be applied without further preparation.

Calibration standards

Arginine
Threonine
Glycin
Alanine
Phenylalanine
Tryptophan
Valine
Leucine

1 mg/mL of each calibration standard, dissolved in water.

Dansylation as described, add 1 mL dansylchloride solution to 1 mL amino acid and fill up to 100 mL with acetone - water 7:3 after derivatization reaction. Concentration of standard solution is 10 ng/µL.

Layer

Precoated HPTLC plates silica gel MERCK 60 F 254, 20 x 10 cm.

Sample application

Bandwise with CAMAG Automatic TLC Sampler III or with Linomat IV; 8 mm bands, 12 mm apart, distance from lower edge 8 mm, 30 mm from the side = 12 samples per plate; delivery speed 15 sec/µL. Apply 5 µL of the samples and different standard volumes.

Application pattern:

S1 U1 S2 U1 S3 U1 S4 U2 S5 U2 S6 U2

S1 = 1 µL = 10 ng absolute U1 = 5 µL (sample diluted 1 : 10)
S2 = 2 µL = 20 ng " U2 = 5 µL
S3 = 3 µL = 30 ng "
S4 = 4 µL = 40 ng "
S5 = 5 µL = 50 ng "
S6 = 6 µL = 60 ng "

S = standard U = sample

Chromatography

Twofold isocratic development with ethylendiaminetetraacetate disodium salt (Titriplex III) - n-butanol - diethylether 1:2:7 in CAMAG Twin Trough Chamber 20 x 10 cm; migration distance 80 mm.

Preparation of developing solvent:

Weigh 5 g ethylendiaminetetraacetate-disodium salt (Titriplex III) into 100 mL measuring flask, add 50 mL dist. water; adjust to pH 9 with NaOH 1n (solution becomes clear); add 10 mL butanol, vortex, add 35 mL diethylether, vortex and use upper phase as developing solvent.

To increase the detection sensitivity place the developed and dried plate in paraffin - n-hexane 2:5; dry with a hair dryer. This procedure enhances the measuring sensitivity by a factor of 3 to 4 compared to an untreated plate.

Densitometric evaluation

With CAMAG TLC Scanner with Labdata System and CATS evaluation software. Scanning by fluorescence at 313 / >460 nm with mercury lamp, monochromator bandwidth 30 nm, slit dimensions 0.3 x 4 mm. Quantitative evaluation with linear regression via peak height.

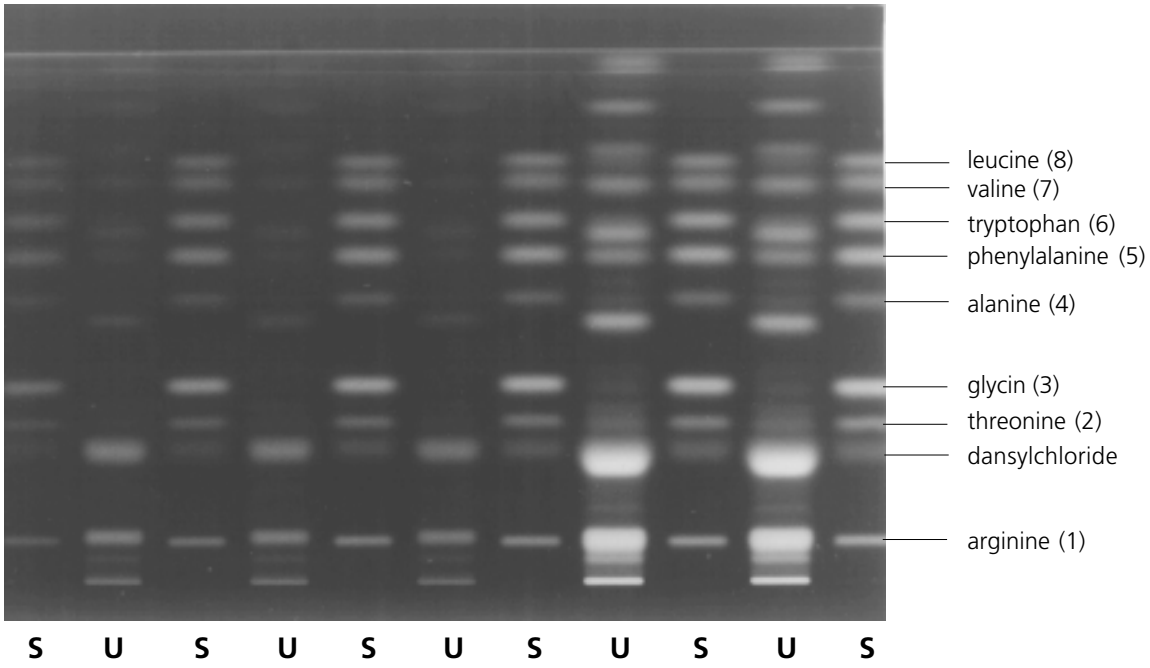

Fig. 1 HPTLC chromatogram of a potato juice sample photographed under 366 nm UV with CAMAG Reprostar II, Polaroidfilm

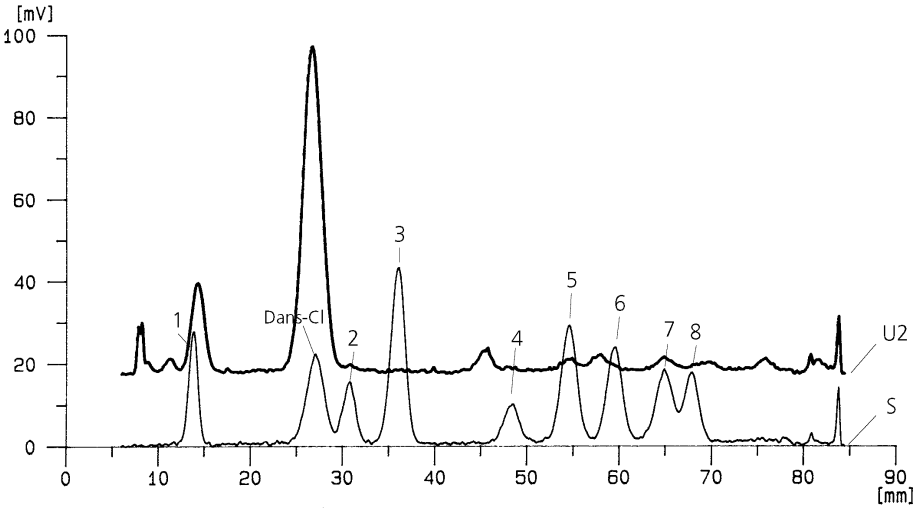

Abb. 2 Densitogram curve of a potato juice sample (U1) and a standard (S)

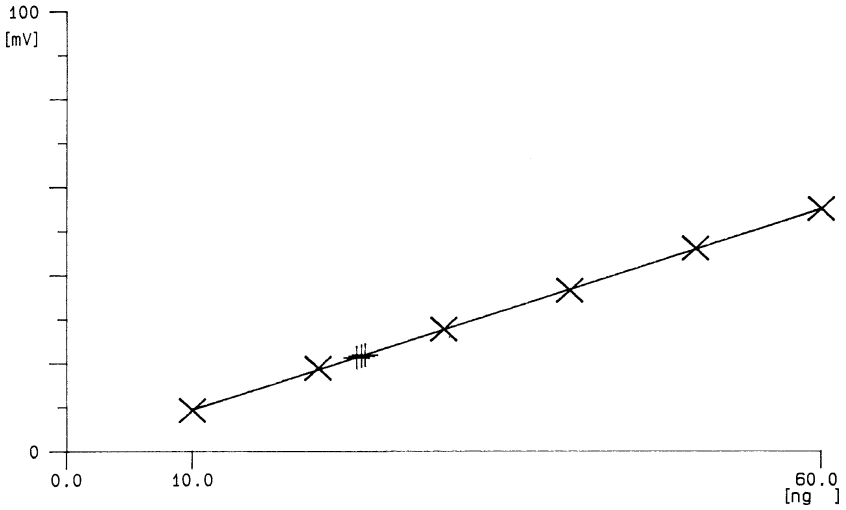

Fig. 3 Calibration curve of arginine (peak height) in the range of 10 to 60 ng

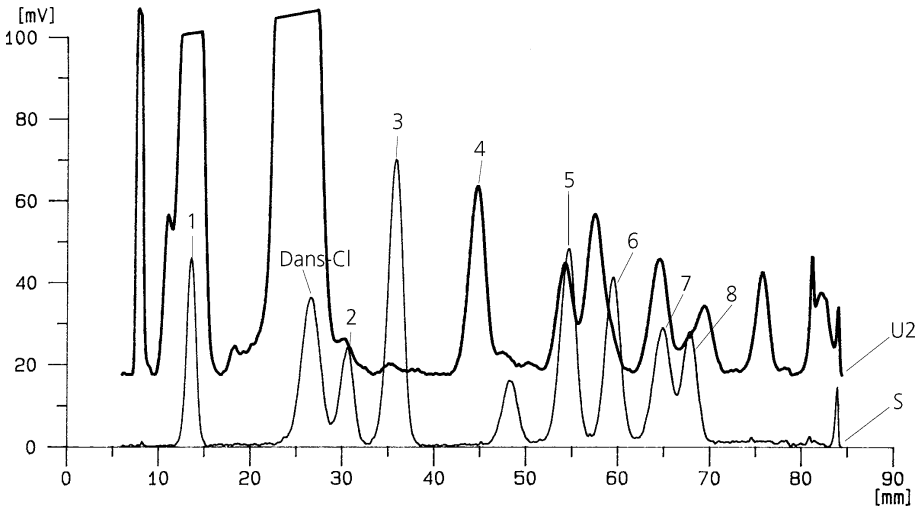

Fig. 4 Densitogram curve of a potato juice sample (U2) and a standard (S)

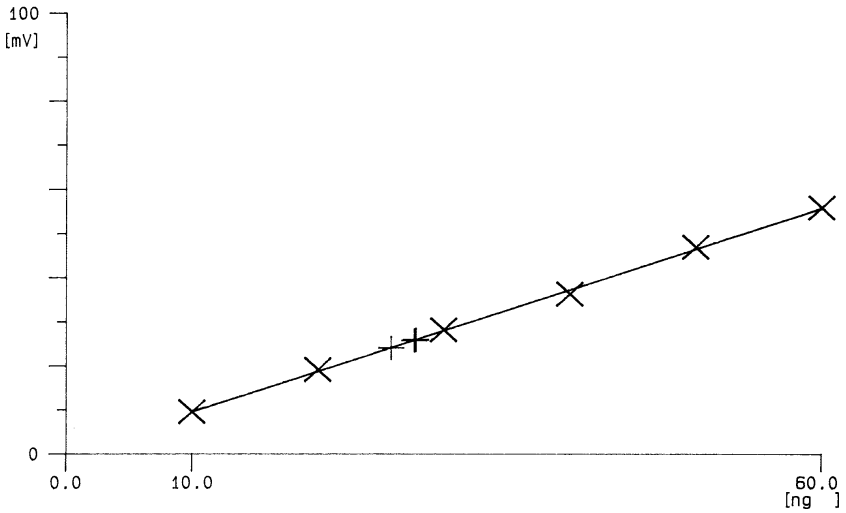

Fig. 5 Calibration curve of phenylalanine in the range of 10 to 60 ng; linear regression via peak height.