CHROMATOGRAPHIC DETERMINATION OF AMINO ACIDS IN PROTEIN HYDROLYSATES

Dome K.V.^(1,2), Bychkov A.L.^(2,3)

(1) Novosibirsk state university
630090, Novosibirsk, Pirogova St., 1

(2) Institute of solid state chemistry and mechanochemistry SB RAS
630128, Novosibirsk, Kutateladze St., 18

(3) Novosibirsk state technical university
630073, Novosibirsk, K. Marksa Ave., 20

Currently functional foods with enhanced bioavailability of important nutrients are of great interest. These foods allows to increase the overall level of consumption of proteins, dietary fiber, vitamins, etc. Sports, children's and medical nutrition products based on low molecular weight oligopeptides and free amino acids are widely used. It is necessary to use methods of qualitative and quantitative determination of the mixture components to optimise the compound of foods. High performance liquid chromatography (HPLC) in gradient elution mode is a promising method for the determination of free amino acids and polypeptides with low molecular weight.

The aims of this work are adaptation of the method of chromatographic qualitative and quantitative determination of amino acids and its aplication for the analysis of protein materials.

Seventeen proteinogenic amino acids were selected as analytes. Phenylisothiocyanate is a high-reactivity reagent for the pre-column quantitative derivatization of amino acids by reverse-phased HPLC. During this work a procedure was developed for obtaining phenylthiocarbomoyl derivatives of amino acids (see the figure). The optimal gradient elution conditions were found to achieve the satisfactory peak resolution ($R_S < 0.5$). Calibration curves were described for all analytes for the quantitative determination of amino acid content.

Derivatisation of amino acids by phenylisothiocyanate

The adapted methodology for the qualitative and quantitative determination has been applied to proteins (for example, bovine serum albumin). The proteins were hydrolyzed by hydrochloric acid to obtain free acids. The quantitative composition of amino acids in hydrolysates changed depending on the time of hydrolysis.

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