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Note

Continuous automatic nitrogen determination for gel chromatography of protein enzymatic hydrolysates

J. AMIOT and G. J. BRISSON*

Département de Zootechnie and Centre de Recherches en Nutrition, Pavillon Comtois, Université Laval, Sainte-Foy, Québec G1K 7P4 (Canada)

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Among the various analytical techniques used for protein determination, only a few have been adapted for the continuous analysis of nitrogenous compounds appearing in the chromatographic elution of protein hydrolysates. UV absorbance measurements have been made at various wavelengths (294 and 280, 280 and 260, 215 and 225 nm), but most of these UV techniques are quantitatively inaccurate when proteins of different molecular weights and of different amino acid composition are studied[1]. Moore and Stein[2,3] based their technique on the reaction of ninhydrin with α-amino nitrogen: unfortunately the intensity of the blue color produced varies for different amino acids; therefore this technique cannot be used for the analysis of the chromatographic elution of protein hydrolysates. The official technique for total determination of nitrogen from proteins of different origin is the Kjeldahl technique[4].

This note describes a technique for the continuous and automatic determination of nitrogenous compounds from a gel chromatography column after *in vitro* enzymatic hydrolysis of proteins.

MATERIALS AND METHODS

To obtain satisfactory chromatographic separations of protein hydrolysates, the following conditions were applied: a proportioning pump (Technicon Proportioning Pump III, Technicon, Tarrytown, NY, U.S.A.) was connected to a flow adapter placed on the top of a 100 × 1.2 cm I.D. column (4200 Precision Column, LKB, Sweden) filled with Sephadex G-25 Superfine (Pharmacia, Uppsala, Sweden) so that a standard 0.02 *M* pH 7.6 phosphate buffer solution[5] continuously flowed through the column. Samples (0.5 ml) of protein hydrolysates were injected at the top of the gel column. The elution flow-rate was 18 ml/h, the void volume was 43.5 ml and the complete elution time about 5 h.

The bottom end of the column was connected to a Digestor (Technicon) with a small capillary tubing (1.0 mm I.D.) and content of eluate was automatically

* To whom correspondence should be addressed.

determined following the Technicon AutoAnalyzer "Kjeldahl Nitrogen" technique[6]. The flow diagram used appears in Fig. 1. Amino–protein nitrogen is converted to ammonium nitrogen by sulfuric acid and selenium dioxide and reacts with alkaline phenol and sodium hypochlorite solutions to produce a compound whose blue color intensity is measured by a colorimeter and recorded. A standard Technicon Auto-Analyzer II equipment was used for all these operations. The analytical system was calibrated with standard ammonium sulfate solutions ranking from 0 to 100 ppm N (mg N/l) before connecting the column to the analyzer. The gel column was calibrated with products of known molecular weight (Fig. 2).

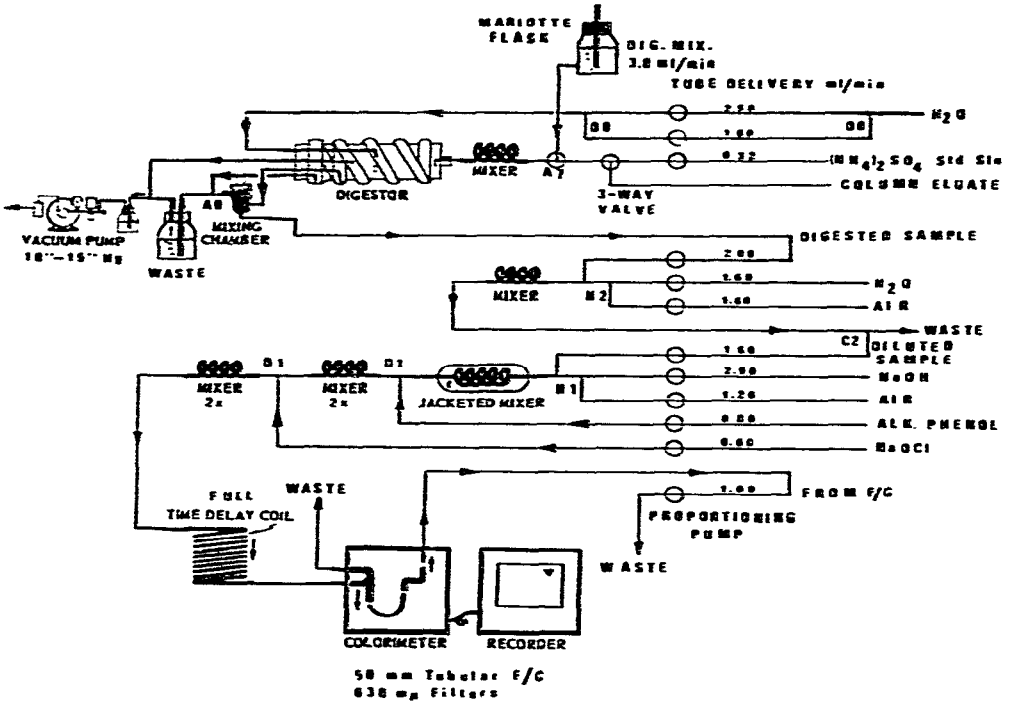

Fig. 1. Flow diagram for the continuous determination of total nitrogen.

RESULTS

With this chromatographic system, a linear relationship was obtained between the time of elution expressed as fractions and compounds of molecular weight varying from 6700 (trypsin inhibitor) to 131 (L-leucine).

This analytical technique has been used for the characterization of the nitrogenous compounds released by *in vitro* pepsin and trypsin hydrolysis of various vegetable and animal proteins. An example of chromatograms obtained with this system is illustrated in Fig. 3. In one case (Fig. 3A) beef protein (25 mg/ml) was

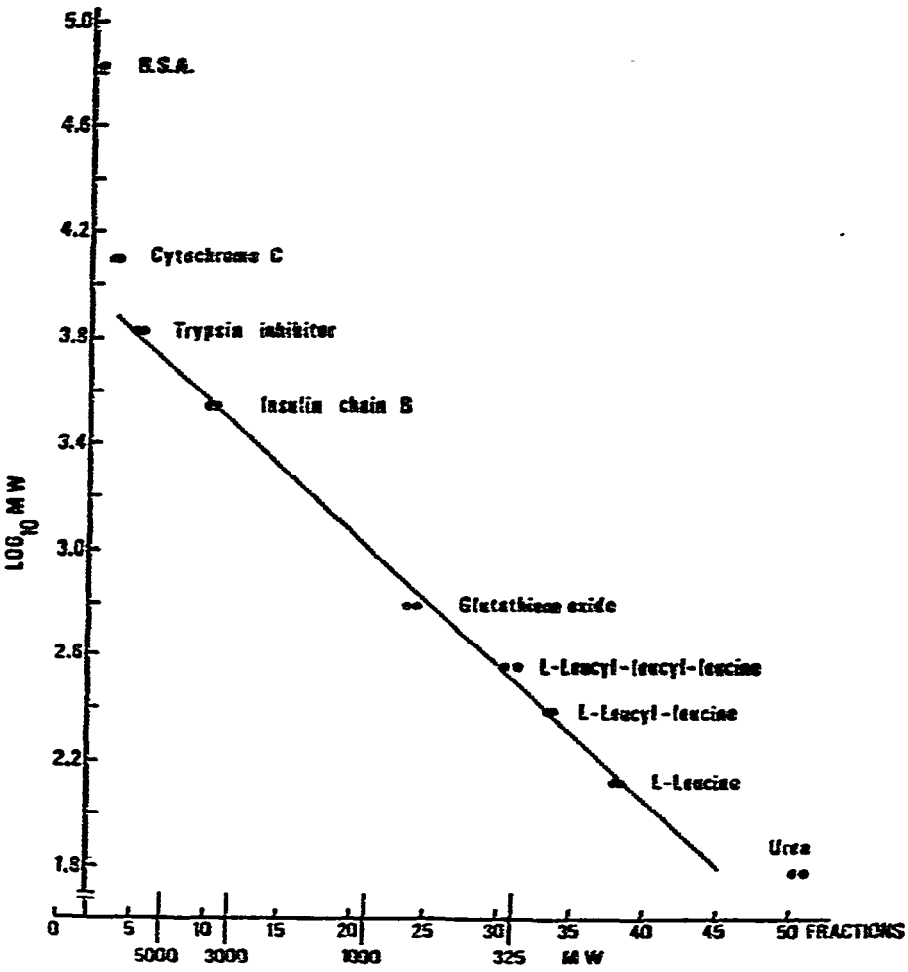

Fig. 2. Calibration curve of the Sephadex G-25 gel column.

hydrolyzed for 20 min with pepsin (1 mg/ml); in the other case (Fig. 3B), the 20-min pepsin digestion of beef protein was followed by a 20-min trypsin (1 mg/ml) digestion.

Satisfactory duplication of the results was obtained and there was no need for more than two or three replications.

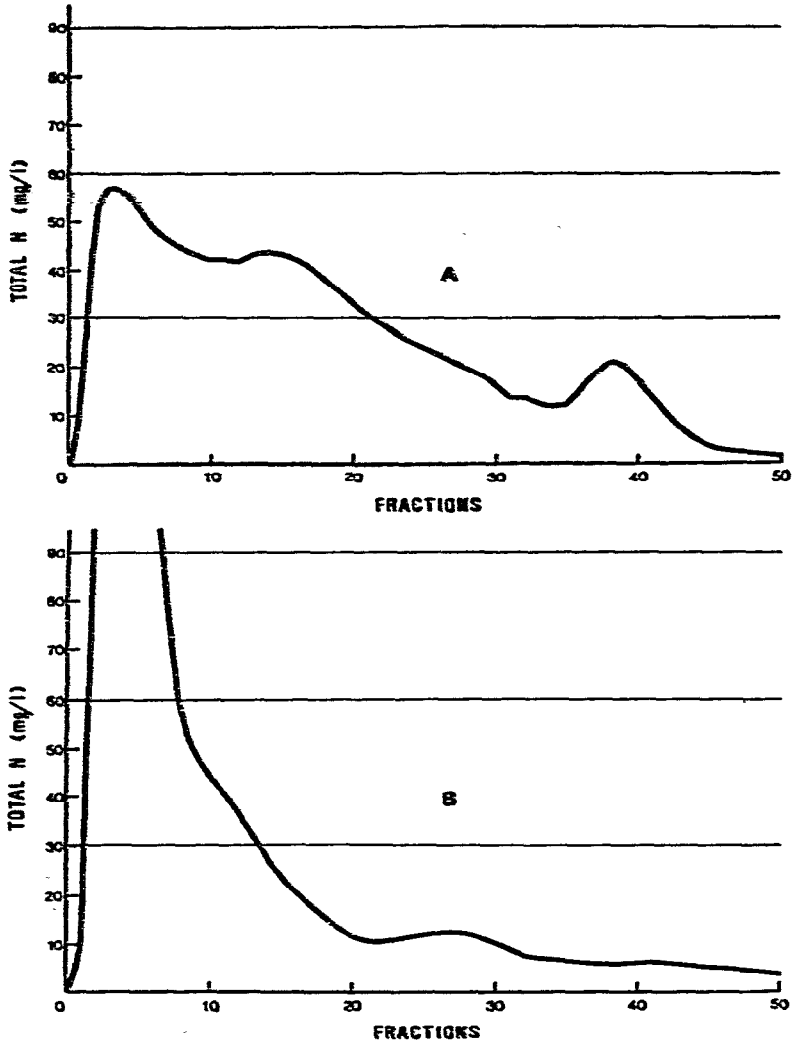

Fig. 3. Chromatogram of beef protein hydrolysates obtained with 20-min pepsin hydrolysis (A) or with 20-min pepsin and 20-min trypsin hydrolysis (B).

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