AN AUTOANALYZER SYSTEM WITH DOUBLE CHANNEL FOR THE DETERMINATION OF MUSCLE PROTEIN AND CONNECTIVE TISSUE PROTEIN

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SUMMARY: Determination of "total protein" content in meat and meat products is not always sufficient because the connective tissue protein content deserves special attention. This paper presents a modification of Arneth's method for the determination of both connective tissue protein and "total protein". Samples of meat and meat products are firstly hydrolysed with H_2SO_4 solution by incubation at overnight at 110°C in an oven. The second step is determination of hydroxyproline in autoanalyser. The same hydrolysed extract is then used for determining the alpha-amino-N-content with 2,4,6-trinitro--benzene-sulphonic acid (TNBS) by the same equipment. TNBS reacts neither with hydroxyproline nor with the N in the aromatic rings of tryptophane and hystidine. Comparison of the TNBS method with the reference method (Kjeldahl) shows that the Kjeldahl method is approximately 5 times more sensitive than the TNBS one. Calculations are carried out with the concepts of Deming's regression. Nevertheless, the results suggest that the "Kjeldahl-convention" may be occasionally substituted with the "alpha-amino-N-convention.

INTRODUCTION: In the protein supply of the population meat is the most important source. Proteins in meat are not uniform, they consist of sarcoplasmic, myofibrillar and connective tissue proteins and in some meat products other proteins of non-meat origin with different nutritive values. In some countries e.g. in the FRG regulations exist concerning the "connective tissue free protein" content of meat products (BEFFE). In this paper an automatic system has been presented in which "total protein" content can be measured parallel to the connective tissue protein content from the sample hydrolysates.

MATERIALS AND METHODS: Raw meat (lean pork, lean beef) and different meat products (sausage of Bologna -type, Italian sausage, canned ham, Hungarian salami etc.) were used as sample units. The principle of connective tissue determination is already well known: hydroxyproline (HOP) can be found only in connective tissue proteins in a relatively high amount and in the case of collagen, in the same proportion. Proteins are hydrolysed with 30 % sulphuric acid solution into amino acids, HOP is oxidised with p-toluene-sulphone-chloramide-sodium (chloramine-T) into pyrrole, and the red colour is developed by using PDAB (Pindur, 1978). The principle of "total protein" content determination with the TNBS method is the following: the primary amino groups of the aminoacids present in the protein hydrolysate, produce a derivate of yellow colour

with TNBS (Okuyama et al., 1960; Satake et al., 1960), that can be measured spectrophotometrically. TNBS does not react with proline and hydroxyproline as these amino acids do not contain primary amino groups.

Determination of connective tissue content

5 g homogenised sample is hydrolysed with 40 cm^3 30 % H_2SO_4 at 110°C for 20 hours. After cooling it is diluted to 200 cm^3. As a basis of comparison, Csiba's manual connective tissue determination was used as reference method (Csiba, 1984). With the automatic method, the same solutions were used as in the manual one: citrate buffer (pH=6,00), 1,5 % chloramine-T solution, PDAB-solution (15 g PDAB + 17 cm^3 70 % $HClO_4$ solution diluted with propanole to 100 cm^3). The oxidising and the colour-developing reagents must be freshly prepared in any case!

Calibration

From a 250 mg HOP/100 cm^3 distilled water stock solution a dilution series of 50; 100; 150; 200; 250 micrograms HOP/cm^3 is prepared.
The analytical modul developed for this purpose is shown in Figure 1., having the following characteristics:

- temperature: 70°C,
- wavelength: 550 nm,
- capacity: 30 samples/hour.

Determination of "total protein" content with the TNBS method

(The analytical modul developed for this purpose is shown in Figure 2.)
Reagents: sodium borate/sodium hydroxide buffer pH=9,50 (Biochemisches Taschenbuch, 1964); 0,18 g TNBS/100 cm^3 in distilled water; HCl-solution 1 mol/dm^3.

Calibration

The H_2SO_4-hydrolysates of bovine serum albumin were used, which were diluted 0-5 times. (Nitrogen content was determined with Kjeldahl's method). The same hydrolysates were used for this test as before (see determination of connective tissue content). This analytical modul is a modification of Arneth's procedure (Arneth, 1983 and 1984).

RESULTS AND DISCUSSION: Comparative measurements for connective tissue determination were made with 40 different sample units in the range of 0,48-8,3 % connective tissue protein content (Figure 3.). Results were evaluated with the regression analysis of Deming (ref.:Körmendy et al., 1989) and the following results were obtained: as Figure 3. shows there is no appreciable difference between the manual and the automatic method. (Details of calculation are not presented here.)
"Total protein" content was determined by the traditional Kjeldahl's and the TNBS method from 50 sample units (lean pork, lean beef, ham, Hungarian salami) and the following results

were obtained:
- the starting point of the regression equation line is in the origin(Figure 4.), however, the slope is different from "1" (γ=0,8312) indicating a proportional bias referred to the bovine serum albumin,
- according to the sensitivity ratio, Kjeldahl's method is about 5 times more sensitive ($S\{y/x\}$ = 0,1864).

At the same time, the standard deviation of the TNBS method (related to the reference method: $s_{\hat{x}}$) is not too high:

$\hat{y}$=0,8312$\hat{x}$ (y= TNBS method; x= Kjeldahl method)

$$s_{\hat{x}}=\frac{s_\varepsilon/\sqrt{2}}{S\{y/x\}} = \frac{0,03048/\sqrt{2}}{0,1864} = 0,1157$$

where
s_ε = standard deviation of the reference (Kjeldahl) method,
$S\{y/x\}$= sensitivity ration of method "y" related to method "x",

$\sqrt{2}$ = denominator of s_ε because each value was the mean of duplicates,

$$cv_{\hat{x}} = \frac{s_{\hat{x}}}{\bar{x}} . 100 = \frac{0,1157}{2,9272} . 100 = 3,95(\%)$$

$$cv_\varepsilon = \frac{s_\varepsilon/\sqrt{2}}{\bar{x}} . 100 = \frac{0,3048}{2,9272} . 100 = 0,74(\%)$$

where
$cv_{\hat{x}}$ and cv_ε are the coefficients of variation of the TNBS and reference methods.
So, the $cv_{\hat{x}}$ value is considerably higher than the cv_ε one, however, the former is still practically acceptable.
The variance of the TNBS method ($v\{\delta\}$) has 2 components (Körmendy et al., 1989):
- a random error component which can be diminished ad libitum by increasing the number of replicates (2 parallel determinations were carried out here with both methods: "x" and "y"),
- a stochastic one which can not be decreased by increasing the number of replicates.

As it can be shown, about 98 % of the total variance of $v\{\delta\}$ is due to the stochastic component in this case.
This is explained by the fact, that the two methods do not measure the same (Kjeldahl → total nitrogen; TNBS → primary amino groups). Figure 5. shows that the specific absorbances of different amino acids are not always the same. Moreover, neither the nitrogen in the ring of amino acids nor one of the α-amino groups of arginine is measured by the TNBS method. The latter is the consequence of degenerated tautomery (Lempert, 1976).

CONCLUSIONS: The automatic method for connective tissue and -amino nitrogen determinations are more rapid and more reliable than the manual procedures the reaction circumstances being constant. Results also suggest that the "Kjeldahl-convention may be occasionally substituted with the "α-amino--nitrogen-convention".

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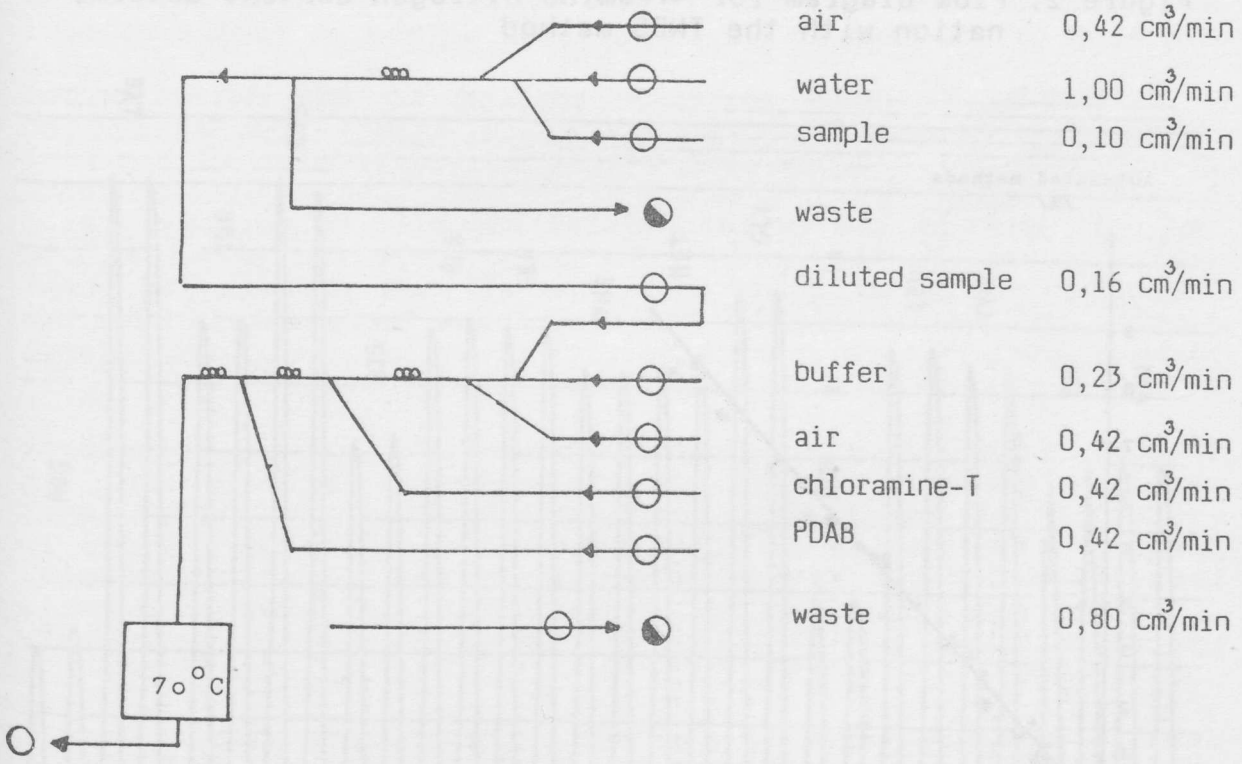

Figure 1. Flow diagram for connective tissue protein content determination

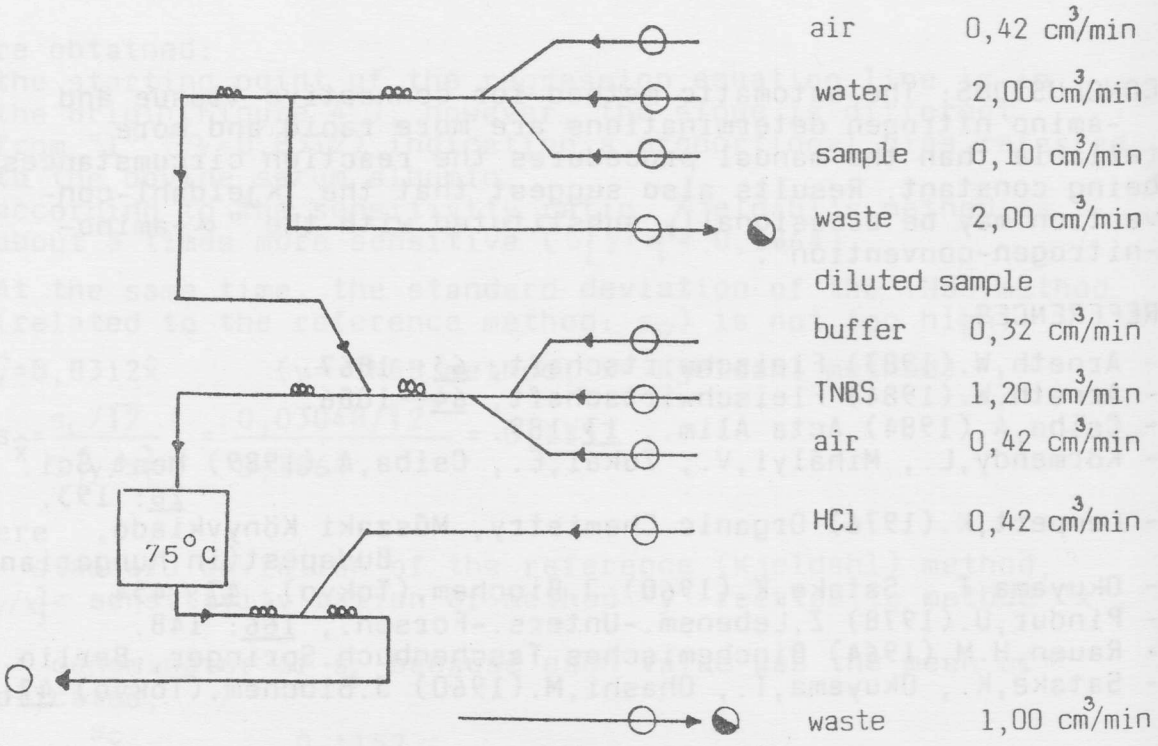

Figure 2. Flow diagram for α-amino nitrogen content determination with the TNBS method

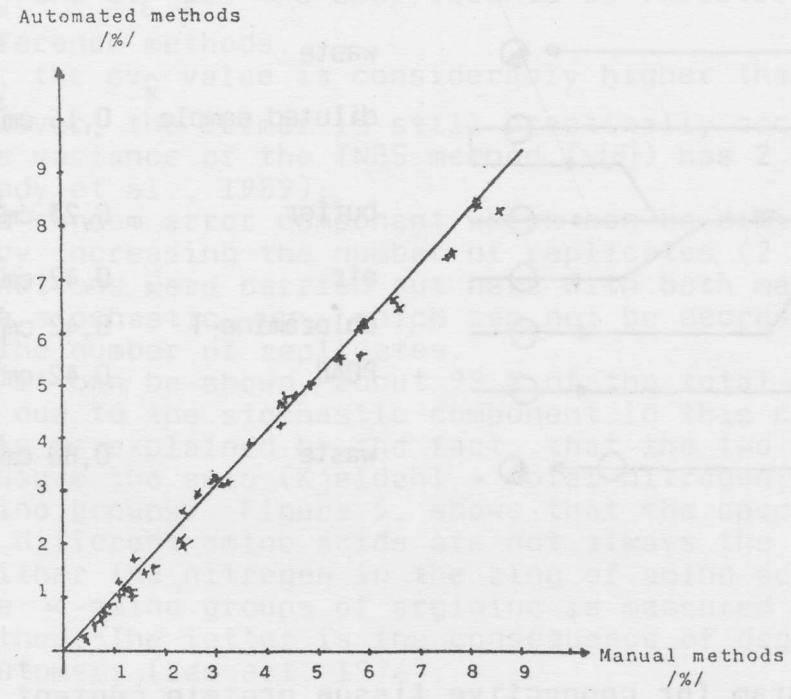

Figure 3. Comparison of manual and automated methods for hydroxyproline determination (g HOP/100 g sample)

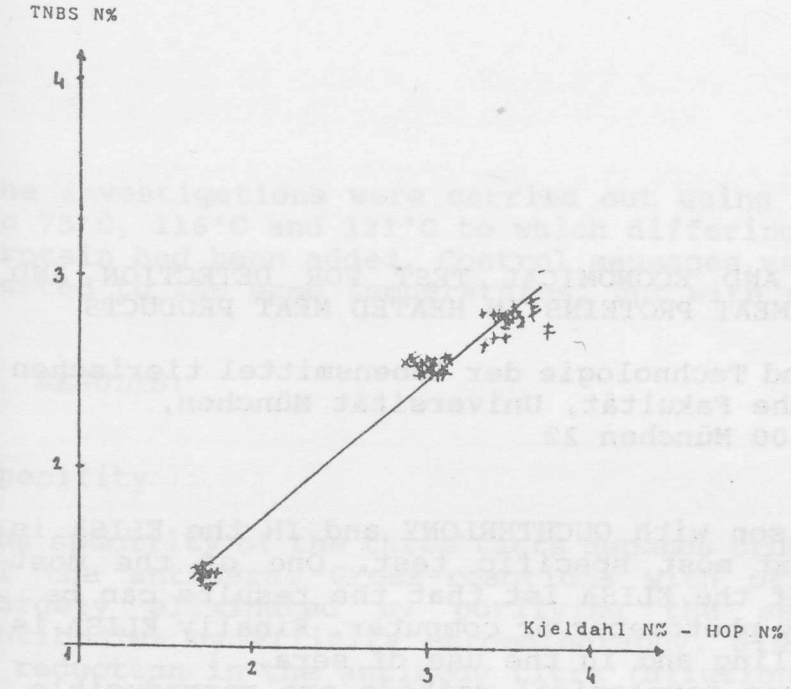

Figure 4. Comparison of Kjeldahl and TNBS methods for "total protein" determination (g N/100 g sample)

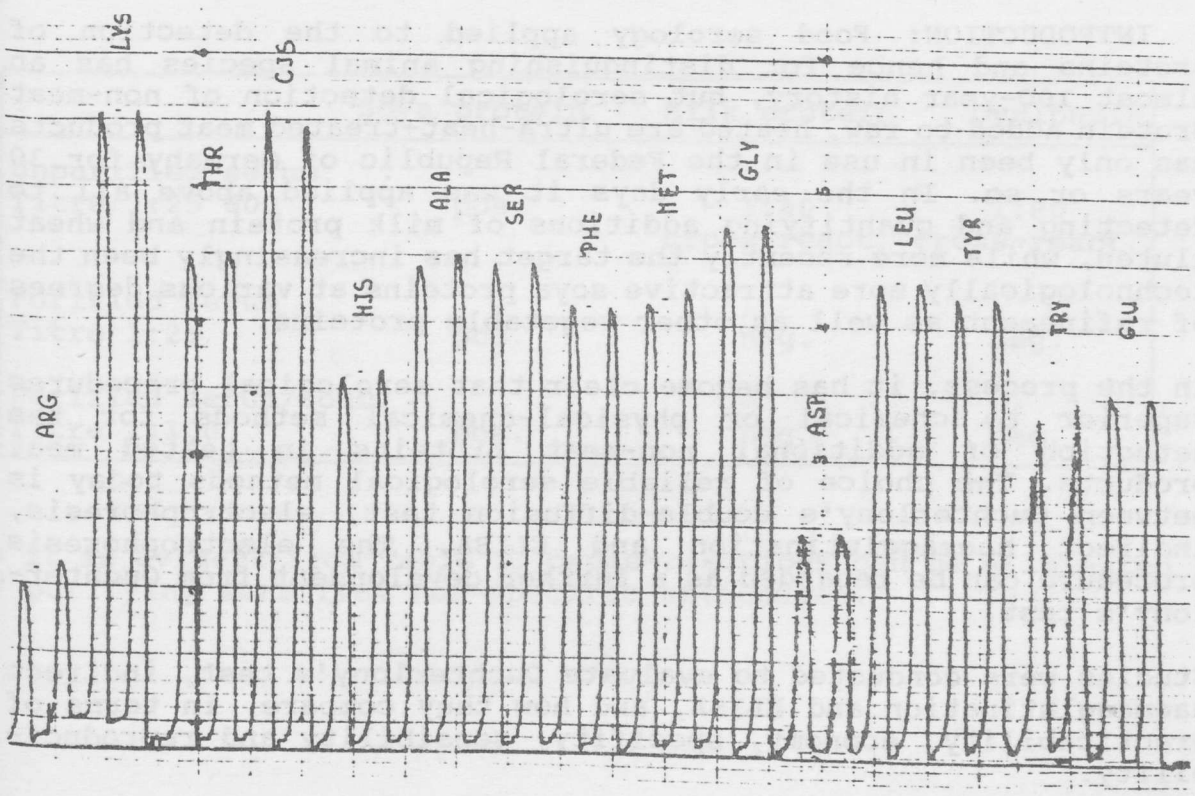

Figure 5. Specific absorbances of different amino acids related to their nitrogen content