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Determination of vitamin B_1 in food by high-performance liquid chromatography and post-column derivatization

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Introduction

Conventional methods of pre-column derivatization of thiamine to thiochrome are time-consuming and susceptible to failure. It is also necessary to determine a sample blind value, as some foods contain substances showing similar chromatographic characteristics as thiochrome [1, 2]. On the basis of methods described previously [1–4] a new procedure for the determination of thiamine in food by high-performance liquid chromatography (HPLC) and post-column derivatization was developed and optimized.

Experimental

The following working conditions have been found most suitable for the determination of vitamin B_1 in food:

Preparation of the food concerned. thiamine is released by heating of the food for 30 min at 120° C with 0.1 mol/l sulphuric acid and enzymatic hydrolysis with Taka diastase at pH 5.0 and 45° C for 15 h [2]. The sample solution is cleaned in an ultra filter and an aliquot part loaded onto the HPLC column.

Conditions for HPLC and post-column derivatization of thiamine to thiochrome. Equipment: 2 HPLC pumps (Waters 590 and 501); Columns: stainless steel 2.5 × 0.4 cm and 12.5 × 0.4 cm packed with LiChrospher RP 18, 5 µm; Thiochrome reactor: Tefzell tubing, knitted, 2 m 1/16″ T-connection for thiochrome reagent supply.

Eluent (methanol + water with ion pair reagent, pH 3.6): mix 250 ml methanol, 0.5 ml 25% ammonia solution and ca. 500 ml distilled water, add 1 g hexane sulphonic acid (Na-salt) dissolve; adjust to pH 3.6 using 1.5 ml of 25% H_3PO_4 solution; fill up with distilled water to 1000 ml and degas.

Thiochrome reagent (0.04% potassium hexacyanoferrate in 1.5% NaOH solution): Dissolve 15 g NaOH (p.A.) in ca. 500 ml distilled water, cool, add 0.4 g potassium hexacyano ferrate (III), dissolve, fill up to 1000 ml with distilled water and degas. The solution, if filled into brown bottles, can be stored for 3 days at room temperature.

Solution for blind value determination: 1.5% sodium hydroxide solution.

Cleaning liquid: distilled water. Flow rate: pump 1: eluant = 1.5 ml/min; pump 2: thiochrome reagent = 1.5 ml/min. Injection volume: 25 µl sample or standard solution (2.0 ng – 20 ng thiamine dichloride). Temperature: 20° C. Thiamine retention time: ca. 5.5 min. Detection spectral fluorometer: excitation wave length = 370 nm, emission wave length = 425 nm. Measurement: Integration of peak area or height.

Note. For connecting the thiochrome reactor to the column exit and to pump 2 for the thiochrome reagent a cyclone mixer (T-connection, PEEK of G. Düster, Saarbrücken) has been found very suitable.

To avoid interfering peaks, it is advisable to rinse the column after separation of the thiamine peak for another 4 min with the eluent. To determine an eventually present blind value, add 1.5% sodium hydroxide solution, instead of the thiochrome reagent, to the eluant (flow rate 1.5 ml/min).

After completion of one analytical series it is advisable to rinse the thiochrome reactor and the detector with distilled water (flow rate: 1.5 ml/min).

Evaluation. The vitamin B_1 content is calculated according to the external standard method. At the beginning and after completion of each HPLC series of measurement, apply 3 injections of a standard comparative solution (thiamine content 2.0 – 20 ng per injection volume) and determine the mean peak area for thiamine. Compare the peak areas of the sample solutions (2 – 3 injections) to those of the standard solutions; make sure that initial weight, aliquot samples and dilutions are taken into account.

Results and discussion

The present method was successfully used to analyze milk and dairy products, meat and meat products, cereals and cereal

Table 1. Vitamin B_1-content of foods analysed by pre- and post column thiochrome derivatisation

Food	µg Vit. B_1/100 g sample				Recovery post-column derivatisation[b]	
	Pre-column derivatisation[a]		Post-column derivatisation[b]			
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Pork, shoulder, raw	711	35	769	12	99	3
Wheat, whole grain	402	5	452	4	98	3
Pumpernickel	55	10	43	2	100	3
Whole milk (3.5% fat)	36	1	39	3	100	3
Potatoes, raw	72	3	70	2	100	3
Baby-food[c]	453	7	458	5	99	3

[a] Method (2)
[b] Described method
[c] Vitaminized (380 µg Thiamine-dichloride/100 g)
$\bar{x}$ = mean value; s = stand. dev.; n = 3

products, vegetables and vegetable products and fruit (about 30 different foods totally).

Blind tests (HPLC separation of thiamine without thiochrome reagent supply) did not show an interfering peak instead of thiamine in any of the foods investigated. Thiochrome present in some foods is separated already after one minute under the present HPLC conditions.

The peak area or -height, respectively, increased linearly as a function of the vitamin quantity in the range of 1–10 ng thiamine per injected volume (40–400 ng/ml). The correlation coefficient was 0.9999. Table 1 shows some results of comparative studies of some selected foods. Accordingly, the thiamine contents determined according to the present analytical method are in the same order of magnitude as the results of precolumn derivatization. The variation coefficients of three repetitions were 1–10%, depending on the kind of food. Recovery rates of thiamine added to the samples were between 98 and 100%.

Conclusion

The present analytical method is a highly specific procedure to determine Vitamin B_1 in food. It is not susceptible to failure, perfectly suitable for serial and routine determinations and yields results which are very well reproducible.

Using a second fluorescence detector (467 nm excitation and 525 nm emission wave length) before the thiochrome reagent supply, vitamin B_2 in foods may be determined simultaneously (retention time for riboflavine ca. 8 min).

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Assay of total- and protein-nitrogen by means of automatic decomposition and coulometric analysis

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Introduction

The analysis of total- and protein-nitrogen is of importance and has been achieved mostly with the well-known Kjeldahl procedure [1]. We improved this procedure with particular regard to thermal digestion and final volumetric analysis with the principle aims of optimal adjustment, miniaturization and simplification of both parts of the analysis to a better practical handling [2]. The absolute principle of the original procedure should be preserved [3].

Principle and methods

Digestion. A new apparatus for digestion [4] carries out parallel sample decompositions with sulphuric acid using an automatic run off. In this way it simultaneously develops and controls graduated ranges of temperature in digestion vessels, so that different temperature gradients are produced. These gradients either act as valves and promote steam-off processes, or they prevent the removal of the decomposition acid. In such a way the control of thermal processes at high temperatures in concise open vessels is reproducibly possible. Thus the digestion processes are accelerated by physical means only.

Catalytic substances or decomposition salts which especially disturb the electrochemical analysis, need not be used. A loss of the initial volume or of concentrated acid (for digestion) does not occur.

In this way, a faultless analysis of nitrogen immediately after the decomposition is possible and the expensive destillation step of the original Kjeldahl procedure can be avoided.

Volumetric analysis. The nitrogen in a defined part of the neutralized mineralizate is estimated by means of the hypobromite procedure [5] using an automatic titrator [6]. The volumetric analysis is done by means of coulometric titration. The automate indicates biamperometrically the redox processes in the measuring cell, currently processes the measuring signals and controls without assistance by means of regulating algorithms the titration until the equivalence point is reached.

The dead-stop-principle finished the reaction.

Hypobromite-procedure:

$$3\,BrO^- + 2\,NH_4^+ \rightarrow N_2 + 3\,Br^- + 3\,H_2O + 2\,H^+ \quad (1)$$

$$3\,Br_2 + 6\,OH^- \rightarrow 3\,BrO^- + 3\,Br^- + 3\,H_2O\,. \quad (2)$$

Electrode-gross-reaction of the reactive element bromine:

$$6\,Br^- \rightarrow 3\,Br_2 + 6\,e^-\,. \quad (3)$$

According to Faraday's law the following electrical quantity of charge results:

$$2 \times NH_4^+ : 36\ g = 6(e^-) \times 96\,494\ As = 578\,964\ As\,. \quad (4)$$

Results and discussion

For the digestion normal reagent vessels are sufficient. They are resistant to concentrated sulphuric acid up to a temperature of +420° C. In these the solid or fluid material (5...1000 µl) of biological origin is placed. For decomposition only sulphuric acid (96%) and for oxidation only hydrogen peroxide has been

Table 1. Nitrogen values of pure di-, tripeptides and amino acids

Substance	Theoretical value [µgN/mg]	Recovery [%]	Relative error [%]
Glycyl-glycyl-glycine	222	97.0	1.1
Alanine	157	96.2	0.8
Glycyl-phenyl-alanine	126	96.8	0.2
Tryptophane	138	97.3	0.4
Glutamine	185	99.9	1.2
Cystine	110	111.0	1.7
Leucyl-glycine	152	90.3	0.9