Beitrag zur Eiweissbestimmung in Fleischwaren

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Die steigenden Ansprüche in Zusammenhang mit dem Eiweissgehalt und dem biologischen Wert von Fleischwaren, sowie eine wirksame Kontrolle und Regulierung der technologischen Prozesse benötigen bessere Eiweissbestimmungsmethoden. Vier verschiedene Eiweissbestimmungsverfahren wurden untersucht bzw. entwickelt: eine verbesserte Kjeldahl-Methode, photometrische Biuret- und Lowry-Methode, eine Farbstoffbindemethode, zwei automatische Verfahren, die auf der Ammoniakbestimmung mit Natriumphenolat bzw. auf der Proteinbestimmung mit dem Folin Reagens beruhen. Aufgrund dreijähriger Erfahrungen mit Eiweissbestimmung in Fleischwaren (Wurstwaren, Schinken, Leberpastete usw.) wurde festgestellt, dass die Biuretmethode allgemein angewendet werden kann. Bei der empfindlicheren Lowry-Methode benötigt man zu den einzelnen Produkten unterschiedliche Eichkurven. Die Farbstoffbindemethode gibt nur zwischen verhältnismässig engen Konzentrationsgrenzen befriedigende Ergebnisse. Die Korrelationskoeffiziente zwischen der Kjeldahl- und den photometrischen Methoden sind in jedem Falle grösser als 0,9. Die Natriumphenolatmethode ist befriedigend und zuverlässig für die automatische Ammoniakbestimmung nach dem Kjeldahl-Verfahren; die Genauigkeit und die Reproduzierbarkeit sind gut. Das Lowry Verfahren ist auch für die Bestimmung der Nicht-Bindegewebe-Proteine verwendbar.

Contribution to the protein determination in meat products

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The increasing demands connected with the protein content and biological value of meat products and also a more effective control and regulation of technological processes needs improved methods of protein determination. Four different methods of protein determination were investigated resp. elaborated: an improved Kjeldahl method, photometric biuret and Lowry methods, a dye binding method, two automated methods based on the ammonia determination with sodium-phenolate reagent resp. direct protein determination using Folin's reagent. On the basis of three year's experience in protein determination of different meat products (sausages, hams, liver pastes etc.) it can be stated that the biuret-method can be most generally used. The more sensitive Lowry method needs separate calibration curves from product to product. The dye binding method gives satisfactory results but only in relatively narrow concentration limits. The correlation coefficients between Kjeldahl method and different photometric methods are in all cases above 0,9. The sodium phenolate method is very satisfactory and reliable for automated determination of ammonia after Kjeldahl procedure. The accuracy and repeatability is good. For the determination of non connective tissue proteins the Lowry method is also applicable.

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Contribution au dosage des proteines dans des produits de viande

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La demande croissante en rapport avec la teneur en protéines et la valeur alimentaire des produits de viande, ainsi que le contrôle et le réglage plus efficace des technologies exigent des méthodes meilleurs pour le dosage des protéines. Quatre méthodes differentes ont été étudiées et développées: une méthode améliorée de Kjeldahl, dosage photométrique de Lowry et avec la réaction du biuret, une méthode de fixation du colorant amido noir-10 B, et deux méthodes de dosage automatique basées sur le dosage de l'ammoniac avec le phénolate de sodium et sur le dosage des protéines avec le réactif de Folin. L'expérience de trois ans a montré que pour le dosage des protéines des produits de viande (saucisses, jambons, pâté de foie etc.) la méthode basée sur la reaction du biuret peut être utilisée le plus généralement. Pour la méthode de Lowry, qui est plus sensible, des courbes d'étalonnage différentes sont nécessaires pour les produits divers. La méthode de fixation de colorant ne donne des résultats satisfaisants qu'entre des limites étroites de concentration. Le coefficient de corrélation entre la méthode de Kjeldahl et les méthodes photométriques est toujours supérieur à 0,9. La méthode de phénolate de sodium pour le dosage automatique de l'ammoniac est satisfaisante; l'exactitude et la reproductibilité sont bonnes. La méthode de Lowry peut être employee même pour le dosage des protéines non provenantes des tissus conjonctifs.

К вопросу определения содержания белка в мясных продуктах.

РАДОМИР ЛАСТЫТЬ, ДЕЖЕ ТЕРЛЕИ И ФЕРЕНЦ ЕРШИ

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Потребность точного определения содержания белка и биологической ценности мясных продуктов а также более эффективные управление и контроль технологических процессов требует более совершенные методы определения белков. Были исследованы и разработаны четыре типа методов определения: ускоренный метод Къельдаля, фотометрические методы с использованием биуретового реагента, и реагента Фолина, красителе-адсорбционный метод и также два методы автоматического анализа /фенолат натрия и реагент Фолина/.

На основе трёхлетнего опыта в определении содержания белка в различных продуктах /ветчины, сосиски, пасты итд./ было установлено что наиболее широко можно применять биуретовый - метод определения белка. Метод Ловриа более чувствительный но требует специальную калибровку для каждого продукта. Адсорбционный метод дает удовлетворительные результаты только в узких диапазонах концентрации белков. Сравнивая метод Къельдаля с фотометрическими определениями получаются коэффициенты корреляции превышающие 0,9.

Натрий-фенолатный метод определения является хорошо воспроизводимым и надежным для автоматического анализа. Метод Фолина можно использовать главным образом для определения белков растворымих в щёлочи.

Contribution to the protein determination in meat products

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Introduction

The increasing demands connected with the protein content and biological value of meat products and also a more effective control and regulation of technological processes needs improved methods of protein determination. The trends of the development of methods for protein determination are characterized with two main directions:

- elaboration of rapid methods for determination
- automation of the analytical work and coupling with the computer technics.

The trends mentioned above are characteristic also for Hungary. In last years many efforts were made for the development of rapid and automated methods. In this paper some of the results of our department are presented.

Materials and methods

Three types of sausages, ham and liver paste produced in Hungary were used as test materials. All the materials were homogenized by fast blade mixer. Usually a 500 g quantity of homogenized product was produced. The extraction of proteins for direct protein determinations was made by following way:

15 g-s of homogenized material were weighed to a 100 ml Erlenmeyer flask. After adding 20 ml of ditilled water and 10 ml of 1 n sodium hydroxide solution a suspension was produced by mixing. The suspension was transferred with the aid of water to a 100 ml volumetric flask, diluted with distilled water accurately to the mark and stored for 12 hours in refrigerator. After this 75 ml (from the upper part of flask) were filtered through a wetter paper filter (Macherey-Nagel 615 1/4 N^0 Ø 15 cm). The first 15 ml-s of the filtrate were discarded. About 50 ml filtered solution was collected.

Determination of protein content by Kjeldahl method. A normal Kjeldahl procedure was used with cc H_2SO_4, potassium sulphate and copper catalyzator. After igestion the ammonia was determined by absorption in boric acid and titrated with hydrochloric acid.

Determination of protein content by accelerated Kjeldahl method. The destruction of organic material was accelerated by adding hydrogen peroxyde.

Photometric determination by Lowry method. 2-5 ml of alkaline extract was pipetted to a 100 ml volumetric flask. 15 ml of 1 m sodium tartrate) were added and filled to the mark with distilled water. Depending on the N-content 0,2 - 10 ml partions of this solution were used for photometric determination. If necessary the volume was adjusted to 10 ml with 1 m Na_2CO_3 : 1 n NaOH : 0,5% $CuSO_4$ (containing 1% KNa - tartrate) = 10:5:1 mixture. After 10 minutes 1 ml Folin reagent was added. After 30 minutes reaction time the optical density of the solution was determined in a spectrophotometer at 750 nm. The N-content was calculated on the basis of calibration curves.

Determination of protein content by biuret method. 2 ml of alkaline extract was mixed with 15 ml of biuret reagent (9,0 g KNa-tartrate, 3,0 g copper sulphate and 5,0 potassium iodide in 1000 ml 0,2 n sodium hydroxide. After 30 minutes reaction time at 37^{o}C the absorbancy of the solution was measured spectrophotometrically at 550 nm. The N-content of the solutio was calculated using calibration curve. Preparation of the calibration curve was based on the determination of protein content of solutions with different protein content using Kjeldahl's method.

Determination of protein content by dye-binding method. 2 ml of the alkaline extract was mixed with 25 ml of dye-solution (0,6000 g Amido-black 10B, 21 g citric acid and 2,5 ml propionic acid in 1000 ml distilled water). After 1 hour resting time, the solution was centrifuged for 10 minutes at 6000 r.p.m. The absorbancy of the clear supernatant was determined

by spectrophotometer at 615 nm. The protein content was calculated using calibration curve.

Determination of protein content by "CONTIFLO" autoanalyser (Typ LABOR MIM BUDAPEST) (Direct Lowry method).

The principal scheme of the CONTIFLO analyser is goven in Fig 1. The arrangement of the analytical module is shown in Fig. 2. The reagent used are the following ones: Folin-Ciocalteu reagent stock solution: 100 g $Na_2WO_4 . 2 H_2O$, and 25 g $Na_2MoO_4 . 2 H_2O$ were dis solved in 700 ml distilled water and 50 ml 85% phosphoric acid and 100 ml hydrochloric acid were added. It was boiled 10 hours using reflux. Half an hour before the end of the boiling 150 g Li_2SO_4 and 50 ml distilled water were added. After cooling the solution was diluted to 1000 ml with distilled water. For the determinations a sixfold diluted solution was used.

Buffer solution: 84 g $NaHCO_3$ and 45 g NaOH were dissolved in 800 ml of distilled water. After cooling it was diluted to 1000 ml.

Copper reagent: 1% KNa-tartrate solution was mixed wits 0,5% $CuSO_4 . 5 H_2O$ solution. A 25-fold diluted solution is used for the determination if the disturbing effect of carbohydrates must be eliminated.

For the determination an alkaline extract containing 0,2 - 1,0 mg protein/ml is used and filled into the sample collector.

Determination of protein content by "CONTIFLO" (Typ LABOR MIM Budapest) autoanalyzer. Indirect determination by measuring ammonia content after Kjeldahl digestion.

The procedure may be used equally after normal Kjeldahl digestion and also after accelerated digestion with hydrogen-peroxide Kjeldahl method. It is important that in the latter case the excess of hydrogen peroxide must be eliminated by boiling. The solution after Kjeldahl digestion must be diluted in this way that the solution shall not contain more than 20 ml of cc H_2SO_4/100 ml. The arrangement of the analytical module is shown in Fig 3. The test solution containing 0,05-0,25 mg N/ml is mixed with sodium hydroxide and diluted with copper sulphate solution. Sodium phenolate and hypochlorite reagents are added. The formed blue indophenol is determined photometrically at 670 nm. The solutions resp. reagents used were the following ones:

Phenol reagent: 14.19 g phenol and 0,1171 g sodium nitroprusside dissolved in 200 ml distilled water and filled to 1000 ml.

Hypochlorite reagent: 6,5 g sodium hydroxide is dissolved in 500 ml distilled water. After cooling 12 ml 5% NaOCl solution is added and filled to 1000 ml.

Standard solution for calibration: Recrystallized ammoniumsulphate is dissolved in distilled water to obtain a solution containing 1 ml/ml nitrogen. The test solutions were prepared by dilution.

Results and discussion.

The Lowry method gives a very good correlation with the results determined by Kjeldahl method. The coefficient of correlation is very high, mostly 0,98-0,99. The method is very sensitive. In solutions containing 0,02 mg/10 ml nitrogen the determination of protein content is possible. The use of very diluted extract has an advantage that the colouring matters extracted from meat-products do not disturbe the determination. Naturally the high degree of dilution is connected with additive sources of lower accuracy. The differences between different products are small.

The biuret-method gives also a good repeatability and accuracy, but a lower sensitivity than the Lowry method. The overall correlation coefficient lies about 0,85, with a standard deviation of $\pm$ 0,5885.

The dye binding method has a disadvantage that the correlation between the protein concentration and dye-binding is only for very narrow limits linear. This fact reduces the applicability of the methods. Nevertheless by given protein concentration intervals the method may be used succesfully. The automatic procedure with "CONTIFLO" autoanalyser based on the Lowry method showed a good repeatability and accuracy. The correlation with Kjeldahl method is good. The differences expressed in absolute protein content are small (0,2-0,7%). Mostly the "CONTIFLO"results are something lower. The differences are probably connected with the connective tissue content of the products. This gives a possibility for the

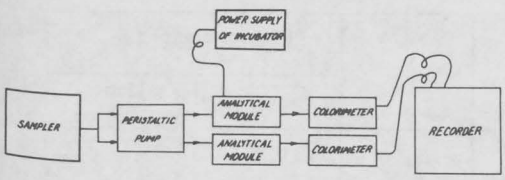

Fig. 1.

Double-channel arrangement of the CONTIFLO continuous flowautomatic analyser

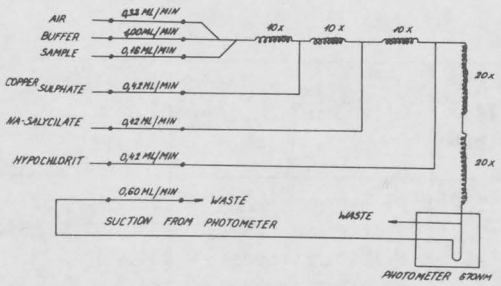

Fig. 2.

Module arrangement for NH_3 determination

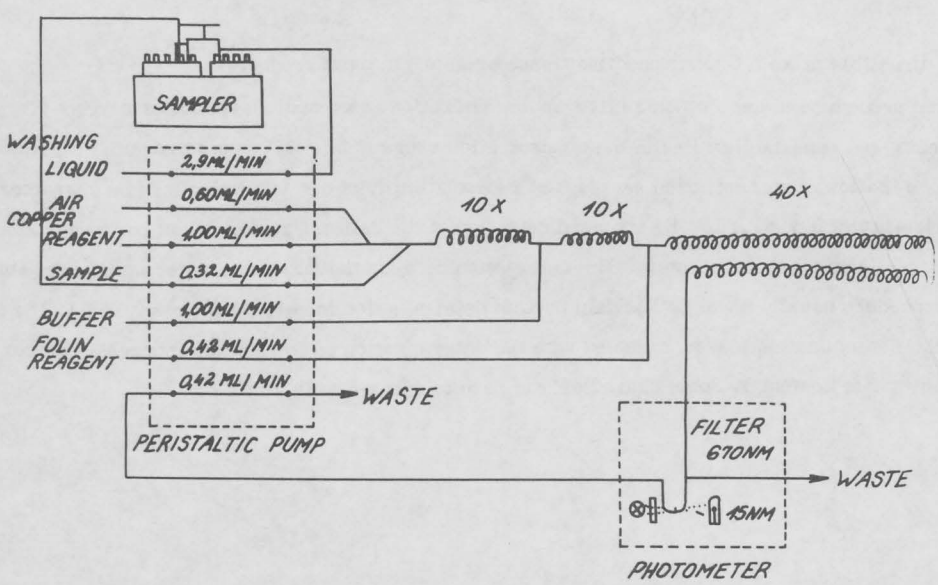

Fig. 3.

Module arrangement for protein determination by Lowry method.

Table 1

Determination of the protein content of meat products
with " CONTIFLO " procedure

N° of sample	Product	Height of peak (mm)	$\bar{X}$	S	Protein content %		Difference (protein %)
					"CONTIFLO"	Kjeldahl distillation	
1.	Frankfurt sausage	51., 51., 52.	51,3	0,06	10,8	-	-
2.	Frankfurt sausage x	53., 52,5., 53.	52,8	0,03	11,1	-	-
3.	Frankfurt sausage x	34., 35., 35.	34,7	0,06	10,8	11,02	- 0,22
4.	Frankfurt sausage	34,5., 35,5., 35.	35,0	1,0	10,92	10,60	+ 0,32
5.	Cured ham	86., 84., 89.	86,3	1,8	18,2	-	-
6.	Cured ham	89., 88., 87.	88,0	1,0	18,5	-	-
7.	Cured ham	88., 89., 89.	88,7	0,4	18,7	-	-
8.	Cured ham	49., 47., 48.	48,0	0,5	15,04	14,90	+ 0,14
9.	Cured ham	52., 51,5., 51,5.	51,7	0,2	16,11	16,33	- 0,22
10.	Cured ham	50,8., 51,5., 51.	51,1	0,3	15,93	15,80	+ 0,13
11.	Luncheon meat	30,5., 31., 30,5.	30,6	0,16	10,64	10,62	+ 0,02
12.	Luncheon meat	28., 28,5., 29.	28,5	0,2	11,25	11,28	- 0,03
13.	Chopped meat	27,5., 27,5., 27,5.	27,5	0,0	9,56	9,63	- 0,07
14.	Oblong cured ham	45., 45., 44,5	44,8	0,16	15,62	15,40	+ 0,22
15.	Oblong cured ham	45,5., 45,5., 45	45,3	0,36	15,72	15,80	- 0,08
16.	Oblong cured ham	46., 46,7., 45,7	46,3	0,34	18.15	18,20	- 0,05

x Lower quantity of digested sample.

determination of digestible protein (non connective tissue protein) in meat products.

The automated procedure of the ammonia nitrogen determination using sodium phenolate reagent gives good results. The optimal linearity and repeatability lies in the concentration range of 50 - 450 ppm nitrogen. Nevertheless the method may be used also in the lower concentration range. The reproducibility of determination may be characterized with a coefficient of variability of 0,9 - 1,2%. The standard deviation of the calibration curve lies between 0,2 - 0,4% protein in a concentration range from 5% to 25% protein. The correlation between the automated determination of ammonia nitrogen and distillation procedure usually made by Kjeldahl protein determination is very good (r = 0,999). The automated nitrogen (ammonia) determination may be compled with the determination of some other inorganic components of meat products (e.g. phosphate content). Some characteristic results are shown in Table 1.