Studies on the content of total and muscle protein in perishable sausages

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An urgent need exists for a rapid,labour-saving,and nonexpensive method for meat protein determination. Until now a few dye-binding procedures have been proposed but not one has been accepted for use on a large scale.
As stated by Torten & Whitaker,1964,the relation between Amido Black 10B bound per g sample and protein content may be expressed by means of a curve,indicating an exponential relation. Later,Moss & Kielsmeier,1967,carefully analysed the reaction between the same dye and meat proteins. They concluded that when the dye-protein ratio was maintained at 0.5:1, no significant change of protein dye-binding capacity was observed. The proposed method is rapid,and easy to perform,but requires a standard curve to be prepared for every particular meat product.
A substantial improvement of meat protein determination by dye-binding was introduced by Pearson,1972.The proposed method may be used for a wide range of protein concentrations,but the author also recommends an individual regression equation to be used for every type of sausages. This requirement limits the applicability of the method when a large number of various sausages and related meat products are to be analysed.
The present paper reports on our attempts to overcome the disadvantages of existing dye-binding methods by simplification of Pearson's procedure and introduction of a common, for all perishable sausages,standard curve using bovine serum albumin (BSA) as a reference substance.

Materials and methods

Twenty-four types of cooked and smoked sausages produced in Bulgaria were used as test material.As many as possible types of sausages from one group were included.
A representive sample of the particular product was homogenized by fast blade mixer (Straume type,USSR) until a fine paste was obtained. The dye-binding procedure used was as follows : Four g of comminuted sample was blended for 1 min (two bursts of 30 s) with about 25 ml of 2M citric acid by means of type MPW-309 Homogenizer,Poland, in a metal beaker. Using 2M citric acid, the obtained suspension was transfered quantitatively into a 50-ml volumetric flask and the volume was made up to the mark. After mixing,exactly 0.5 ml of the resulting suspension was pipetted into a centrifuge tube and 1 ml of chloroform was added.The mixture was shaked vigorously for 1 min ; then 5 ml of dye solution (0.21 g Amido Black 10B,Merck, in 450 ml 0.1 M citric acid) was added and the mixture was shaked for another minute. After short centrifugation,exactly 1 ml of the supernatant was diluted by pipetting into 50 ml of destilled water. Blank was prepared by diluting the stock dye solution 56-fold with water. The optical density of both sample (D_s) and blank (D_b) was measured in 1-cm cell at 615 nm against water.The difference (D_b-D_s) was calculated and expressed in mg dye bound.The absorption coefficient of the Amido Black 10B used in our experiments was a_{620}=77.8 $cm^{-1} g/l^{-1}$
Nitrogen determination was performed as recommended in ISO 937-1978 (Kjeldahl method), and the results expressed as protein(N x 6.25).
Bovine serum albumin (BSA) was purchased from Fluka(Switzerland).Its protein content(N x 6.25) determined by the ISO method and solutions with the required concentrations were prepared using saline.

Results and discussion

As can be seen from Table I the protein content (N x 6.25) of all types of analyzed sausages ranges from about 10.5 to 13.5 %.If the established standard deviations are taken into account this range will widen - from about 9.0 to 15%.Provided a normal distributions are assumed this range will include at least two thirds of all analyzed samples. Using dilutions of sausage emulsions we have shown that the requirement for a linear relationship between the dye bound and protein concentration in this particular range is met without any changes of the described procedure (Nestorov et al.,1980).
The method used gives a very good correlation with the results determined by the Kjeldahl method. The correlation coefficients are high,mostly over 0.90,at significant at P 0.05. However,as it could be expected,the calculated regression equations vary greatly from one type of sausage to another,apparently depending on their specific composition. Although once established,the equations could be used for practical purposes,this approach would be always a source of errors and confusion.
We think that the only outcome of this situation is to introduce a common standard curve for all sausage types. This is feasible if a reference protein can be found whose dye-binding capacity (DBC) is commensurable with the average DBC of meat products proteins.
The fifth column of Table I shows the DBC values calculated from the means of protein content and the respective quantity of bound dye. With a few exeptions no large differences among various types of sausages were observed. Dye-binding capacity of a particular protein depends on its basic amino acids content and represents its unique characteristic. Hence when a mixture of proteins is concerned its DBC might be predicted from the respective DBC values of all individual proteins,or those of the most important protein groups.
Nitrogen containing compounds of meat products are usually divided into four groups : sarcoplasmic,myofibrillar,and stromal proteins,and non-protein nitrogenous substances. Their experimentally determined DBC values are 0.44,0.57,0.06,and 0.00,respectivelly.We have also

established that heat treatment at 80°C for 90 min does not change significantly the DBC of "raw" myofibrillar and sarcoplasmic protein fractions, but at least 2-fold increase is observed when the stroma fraction is treated under the same conditions (unpublished results).

A rough est mate of non-protein nitrogenous compounds content ranges from 10 to 15% of all N-containing substances present in meat products. Assuming the relative content of these non-protein compounds does not vary significantly from one type of sausage to another, one can calculate DBC of the proteinaceus matter. The respective value calculated from our results (Table I) is about 0.440 - 0.460. This coincides well with the DBC of bovine serum albumin (BSA), so the latter was chosen as a reference protein.

The standard line derived from the regression equation calculated on the basis of BSA is depicted in Fig.1. It was used to convert the (D_b-D_s) values obtained from the analysed sausages into percent protein.

Apparently one must specify the use of the term "protein content". As is generally accepted "protein content" means nitrogen content, determined by Kjeldahl' procedure, multiplied by a conversion factor of 6.25. This includes however not only proteinaceus matter but also all non-protein N-containing compounds. Moreover, since conversion factors for meat proteins and gelatin (collagen) are 6.25, and 5.50, respectivel, this causes additional inaccuracy when low-quality meat cuts are used for sausage preparation.

On the other hand, we claim that protein determined by our procedure is a better estimate of the true muscle protein content. For this reason we adopted the term "muscle protein content" in order to differenciate it from "protein content" determined by Kjeldahl's procedure. Practical tests which are underway now show that this approach could prove to be of benifit for both the manufacturer and the consumer.

References

(1) Moss, V.G. & E.W. Kielsmeier /1967/ Food Technology 21 : 33A-36A
(2) Nestorov, N., Dilova, N., Grozdanov, A., Petrova, D., Gateva, A. /1980/ Meat Industry Bulletin XIII (1) 7 - 12.
(3) Pearson, D. /1972/ Food Manufacture 47 : 45 - 47
(4) Torten, J. & Whitaker, J.R. /1964/ J. Food Science 29 : 168 - 174.

Table I. Protein content and dye-binding capacity of 24 types of perishable sausages

Sausage type	Bound dye (mg dye/2 g product)		Protein content (N x 6.25)		Dye-binding capacity (mg dye/mg protein)	Correlation coefficient * P<0.05 ** P<0.01 *** P<0.001	Regression equation
	$\bar{x}$	S.D.	$\bar{x}$	S.D.			
A. Small sausages - Frankfurter type							
(1)	1.013	0.07	12.7	0.9	0.399	0.87*	y = 10.7x + 2.6
(2)	0.765	0.11	10.8	1.2	0.354	0.97***	y = 11.7x + 0.7
B. Small sausages - Wienner type							
(3)	0.910	0.05	11.9	0.9	0.382	0.76*	y = 9.0x + 3.7
(4)	1.133	0.12	14.4	1.0	0.393	0.95*	y = 9.6x + 3.4
(5)	0.985	0.12	12.8	1.4	0.385	0.91*	y = 10.4x + 2.5
(6)	0.971	0.14	12.0	1.3	0.405	0.96**	y = 9.1x + 3.1
(7)	0.958	0.15	12.6	1.3	0.380	0.95***	y = 8.7x + 4.3
C. Liver sausage							
(8)	0.955	0.68	12.7	1.0	0.380	0.82*	y = 12.1x + 1.1
D. Meat loaf							
(9)	0.917	0.12	11.9	1.4	0.385	0.95***	y = 10.8x + 1.9
E. Large sausages - Fine cut - emulsion type							
(10)	0.834	0.06	11.2	0.5	0.372	0.85**	y = 7.7x + 4.8
(11)	0.939	0.11	11.9	1.0	0.395	0.91***	y = 8.0x + 4.4
(12)	0.934	0.14	11.7	1.4	0.399	0.99***	y = 10.0x + 2.3
F. Large sausages - Fine cut with ground or chunk meat added							
(13)	0.946	0.11	13.0	1.5	0.364	0.83*	y = 11.4x + 2.1
(14)	0.918	0.07	14.0	0.8	0.328	0.78*	y = 9.8x + 5.0
(15)	0.909	0.12	11.3	1.4	0.402	0.96**	y = 11.2x + 1.1
(16)	1.006	0.11	12.2	1.4	0.412	0.95**	y = 11.8x + 0.3
(17)	0.975	0.04	12.4	0.7	0.393	0.83*	y = 14.7x - 2.1
(18)	0.960	0.11	12.7	0.9	0.387	0.96***	y = 8.3x + 4.7
(19)	1.030	0.09	13.1	0.8	0.393	0.85**	y = 7.3x + 5.5
(20)	1.045	0.11	13.4	0.9	0.390	0.79*	y = 6.3x + 7.0
(21)	0.952	0.15	12.2	1.6	0.390	0.99***	y = 10.6x + 2.0
(22)	1.038	0.15	13.1	1.5	0.396	0.96***	y = 9.2x + 3.5
(23)	1.002	0.11	12.9	1.3	0.388	0.79**	y = 9.5x + 3.4
G. Large sausages - Coarse cut							
(24)	0.869	0.06	12.0	0.9	0.362	0.86*	y = 10.3x + 3.1

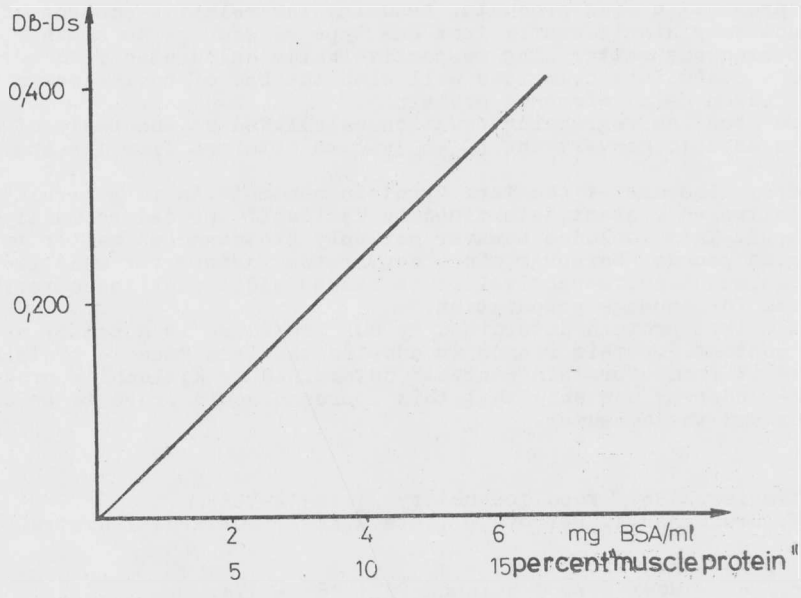

Fig. 1. Comparison of bound dye ($D_b - D_s$ at 615 nm) with Kjeldahl values (N x 6,25) for bovine serum albumin (BSA)