8:8 Possibilities for connective tissue-free muscle protein determination in cooked sausages by a dye-binding method

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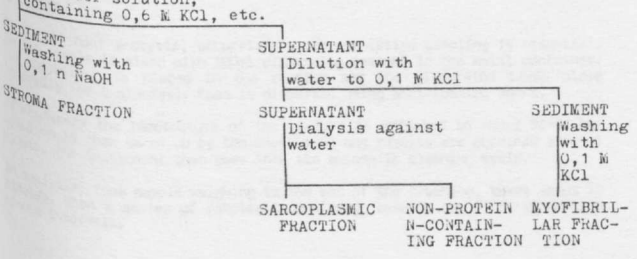
As reported previously (Dilova et al., 1981), a modified version of Pearson's dye-binding method (Pearson, 1972) had been developed for the simple and rapid protein content determination in meat-only cooked sausages. The major steps of the procedure used were as follows: A portion of the ground product, containing 350-500 mg protein (N x 6,25), was blended with 50 ml of 2M citric acid. After filtering through cheese cloth, exactly 0,5 ml of the resulting suspension were transferred to a centrifuge tube. The possible interference by the lipids present was avoided by adding 1 ml of chloroform. Five ml of the Amido Black 10B solution were added and the mixture was shaken vigorously for 1 min. The precipitate formed was separated by a short-time centrifugation, then exactly 1 ml of the clear supernatant was diluted by pipetting into 50 ml of distilled water. A blank was prepared by diluting the stock dye solution 56-fold with water. The optical density of both sample (OD_s) and blank (OD_b) was measured at 620 nm against water. The difference ($OD_b - OD_s$) is an estimate of the dye lost and can be related positively with protein content.

Dye-binding capacities (DBC) of the nitrogen-containing fractions

To ascertain the dye-binding capacities of the different nitrogen-containing components of muscle tissue, the three major protein fractions, as well as the non-protein nitrogenous fraction, were extracted as follows:



The nitrogen content of the fractions was determined by Kjeldahl's method. A factor of 6,25 was used to calculate protein from nitrogen in all fractions except for the stroma fraction. For the latter fraction, the factor used was 5,55.

Aliquots of each fraction solution or suspension, containing ca. 2 mg protein, were allowed to react with 5 ml of Amido Black 10B (Amidoschwarz 10B, Merck, $a_{620} = 73,3\ cm^{-1}\ (g/L)^{-1}$) solution containing 2,33 mg of the dye, for 1 min with continuous shaking. After centrifuging at 3000 rpm for 5 min, aliquots of the clear supernatant were diluted 51-fold with distilled water and measured at 620 nm against water. The mean estimated dye-binding capacity values, expressed as mg bound dye per mg protein, are shown in the following table:

	Fractions			
	Myofibrillar	Sarcoplasmic	Stroma	Nonprotein nitrogen
In the raw state	0,57(0,07)*	0,44(0,04)	0,06(0,01)	0,0
After cooking**	0,56(0,09)	0,44(0,03)	0,16(0,03)	0,0

* Mean (S.D.); n = 5; ** 80°C for 60 min.

Seperich and Price (1979) also found differences between various nitrogen-containing fractions with respect to their capacities to bind Orange G. The ranking order was essentially the same as we found with Amido Black 10B: Myofibrillar > Sarcoplasmic > Stroma.

As shown in the preceding table, heat treatment (80°C for 60 min) did not change the DBC of myofibrillar and sarcoplasmic fractions but at least doubled the DBC of the stroma fraction. Apparently, the swelling and partial solubilization of collagen facilitated dye-binding through the exposure of more binding sites. For comparison, microbiological grade gelatine binds 0,38 mg dye per mg protein (N x 5,55).

Dye-binding capacity of the proteins of cooked sausages

Proteins constitute 16-22% (mean 18,5) of the muscle mass. Non-protein nitrogenous substances account for another 1,5%. As evidenced by many investigators, the contribution of collagen, the major component of connective tissue, to the total protein content of meat varies greatly among different cuts. For instance, Voznarova et al. (1968) found that the collagen content of different cuts of veal and beef varied from 3 to 24 g per 100 g protein, and Board et al. (1978) reported that cured pork shoulder and canned ham had a collagen content, as a percentage of total protein, of 7-17%, and 6-11%, respectively. Our investigations showed that the connective-tissue protein contents (hydroxyproline x 8,07) of beef and pork raw materials, as well as of various cooked sausage products were as follows:

	n	% total mass		% total protein	
		Range	Mean	Range	Mean
Pork (8% fat)	20	0,85 - 1,18	1,01	4,3 - 6,5	5,65
Pork (37% fat)	20	1,24 - 1,46	1,36	10,3 - 12,0	11,15
Pork (55% fat)	20	1,18 - 1,31	1,25	13,6 - 15,0	14,30
Beef (5% fat)	10	1,62 - 2,52	2,07	8,9 - 13,0	10,5
Beef (8% fat)	10	2,25 - 3,30	2,77	12,0 - 17,0	14,5
Cooked sausages	22	1,05 - 2,69	1,67	6,0 - 23,0	15,5

To calculate the expected DBC values of cooked meat products with different connective tissue content, we assumed a constant ratio between the major nitrogen-containing fractions with the exception of stromal proteins, myofibrillar : sarcoplasmic : non-protein nitrogenous = 1,9 : 1,2: 0,3 (Forrest et al., 1975), and the percentages of connective tissue protein content (% of total protein) varying from 0 to 25%. The DBC values used were as mentioned above for the respective nitrogenous fractions after cooking. The calculations produced the following DBC values:

For 0%	connective	tissue	content,	0,464	mg bound dye/mg protein
" 5%	"	"	"	0,457	" "
" 15%	"	"	"	0,426	" "
" 25%	"	"	"	0,395	" "

Experimental data involving three different types of cooked sausages (n = 22) showed values of 0,398 ± 0,030, and 0,472 ± 0,021 (mean ± S.D.) for the DBC of the total protein (N x 6,25) fraction and the collagen-free protein fraction, respectively. Apparently, there was a good agreement between the experimental and the expected values.

Owing to the relatively small DBC value of the stroma protein fraction, even after heat treatment, it contributes only a little to the total DBC of the sausage homogenate. While the connective tissue nitrogen comprises up to 25% of the total nitrogen content, its part in the total DBC is as little as 2%. Myofibrillar and sarcoplasmic proteins account for the remaining 98%. Therefore, the quantity of dye lost when the suspended proteins react with Amido Black 10B under the above specified conditions could be used as a direct measure of the collagen-free nitrogen content, provided a suitable conversion factor or reference protein(s) be chosen.

Two possible approaches to the standardizing of the dye-binding method

The first approach consists of the usage of a single numerical factor for the direct conversion of the dye bound, as measured by the decrease of absorbance at 620 nm, into per cent connective tissue-free muscle protein (CTFMP) content. This implies the dye itself should be used as a reference substance provided that the DBC value of the product(s) of interest is generally recognized and accepted. Such an approach necessitates, however, a steady supply of a high purity dye or, at least, of a uniform purity one.

The properties of the four different samples of Acid Black-1 (C.I. 20470) studied in this laboratory are listed in the following table:

Sample	Brand name	Source	a_{620nm}*
1.	Amidoschwarz 10B	Merck (FRG)	73,3
2.	Amido Black 10B	Reanal (Hungary)	77,7
3.	Amidoschwarz 10B	Chemapol (CSSR)	51,1
4.	Naphthol Blue Black 12B	BDH (England)	43,5

* $a_{620nm} = A\ cm^{-1}\ (g/L)^{-1}$

The absorbtion coefficients listed indicate that a great variability exists in the dye content among different samples. Many commercial brands of Acid Black-1 (Amido Black 10B) are known to contain less than 60% dye, large amounts of sodium chloride, and variable amounts of several red or orange dyes. Thus we are not able to recommend any particular supplier or brand of dye. Apparently, the first approach, even though promising, is still inapplicable. For practical purposes, however, it is advisable to standardize the dye solution utilized by daily checks of the absorbance of the appropriate diluted samples.

The second possible approach implies choosing reference protein(s), the DBC of which is close to that of the total connective tissue-free proteins in the sausage homogenate. Various blood proteins which are commercially available were tested for their capacity to bind Amido Black 10B. The results obtained were as follows:

Protein(s)	DBC (mg dye bound/mg protein)
γ-Globulins rabbit (Koch-Light Lab., GB)	0,223
α-Globulins bovine (Koch-Light Lab., GB)	0,235
α-Globulins porcine (Koch-Light Lab., GB)	0,350
Albumin rabbit (Koch-Light Lab., GB)	0,530
Albumin, bovine serum (Fluka)	0,480

Bovine serum albumin /BSA/ (Cohn fraction V) was chosen as a reference protein as its DBC is very close to the experimentally estimated DBC of the collagen-free protein fraction (0,473 ± 0,021). Further, the standard line derived from the regression equation calculated on the basis of BSA was used to convert the ΔOD_{620nm} value into % CTFMP content.

To check the reliability of the proposed standardization, 20 samples of cooked sausages with different connective tissue content

were analysed for total protein (N x 6,25), CTFMP content (BSA-equivalent), and connective tissue content (hydroxyproline x 8,07). Results are shown in the following table:

Sample	% Total protein (N x 6,25)	% CTFMP (BSA-equiv.)	% Connective tissue protein (OHPro x 8,07)	(2)+(3)	(1)-(4)
	(1)	(2)	(3)	(4)	(5)
1	12,97	11,2	1,20	12,40	+0,57
2	13,87	12,80	1,40	14,20	-0,37
3	11,92	10,0	1,47	11,47	+0,45
4	13,89	13,6	1,15	14,75	-0,86
5	12,90	11,6	1,20	12,80	+0,10
6	12,41	11,8	1,40	13,20	+0,79
7	12,67	10,7	1,15	11,85	+0,82
8	17,06	16,2	1,52	17,72	-0,66
9	13,31	11,7	1,27	12,97	+0,34
10	11,59	10,3	1,08	11,11	+0,48
11	13,56	12,60	0,96	13,56	0,0
12	13,67	11,9	1,47	12,37	+1,30
13	12,90	10,6	1,06	11,66	+1,24
14	14,65	13,0	1,62	14,62	+0,03
15	12,75	10,4	1,19	11,59	+1,16
16	13,56	10,3	2,44	12,74	+0,82
17	13,89	12,6	1,15	13,75	+0,14
18	13,50	12,0	1,83	13,83	-0,23
19	13,94	11,4	1,94	13,34	+0,60
20	11,95	10,2	2,17	12,37	-0,42
n = 20					$\bar{x}$ = +0,26 S.D. = 0,66

It was expected that the results of total protein determination (column 1) were not significantly different from the sum obtained on adding % connective tissue protein to % CTFMP (column 4). To test $H_o : \bar{x} = 0$ against $H_A : \bar{x} \neq 0$, we calculated Student's t ($t_{calc.} = 1,73$) Since the tabulated value of t for 19 degrees of freedom (P = 0,05) is 2,09, we could not reject the null hypothesis. Apparently, we had no evidence to conclude that the proposed dye-binding method had over- or underestimated the actual values of connective tissue-free muscle protein content in the cooked sausages tested.

References

1. Board, P.W., Montgomery, W.A. & Rutledge, P.J. (1978) J.Sci. Food Agric. 29:569.
2. Dilova, N., Grozdanov, A., Nestorov, N., Petrova, D., Dikova, G., Tabakova, R. (1981). Proc. 27th EMMRW, Vienna, Paper F:7.
3. Forrest, J.C., Aberle, E.D., Hedrick, H.B., Judge, M.D. & Merkel, R.A. (1975). In: "Principles of Meat Science", W.H. Freeman & Co., USA.
4. Pearson, D. (1972) Food Manufacture, 47:45.
5. Seperich, G.J. & Price, J.F. (1979) J.Food Sci., 44:643.
6. Vognarová, I., Dvorák, Z., Böhm, R. (1968) J.Food Sci., 33:339.