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Some experience with a rapid protein determination in meat

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INTRODUCTION

For a good supervision of the production of meat products in the meat industry it is desirable to have, at any moment, the disposal of data concerning the water, fat and protein content of raw materials, sausage emulsions and finished products.

At the moment it is possible to determine the water and fat content of meat in a rather short time (1, 2).

The usual method for the determination of the protein content in meat, the Kjeldahl method, is not suited for a rapid determination on a large scale. To meet this difficulty Meester and Houtepen (3) developed some years ago a more rapid method for the protein determination in meat. They utilized the property of acid dyestuffs, for example Cochineal red A, to form a precipitate with proteins in an acid solution. By estimating colorimetrically the concentration of the dye before and after the reaction with the proteins, it is possible to calculate the amount of protein, added to the dye solution.

Since 1961 a large number of protein determinations in meat has been carried out in our institute. The purpose was to examine the relation between the protein content, according to Kjeldahl, and the amount of the dye bound by a specified quantity of meat, determined by the dye-binding method.

Up till now 872 samples of beef, veal and pork were analyzed using both methods and the relation between the results of these methods was calculated.

As appears from the following results of this investigation, we found a good correlation between the protein content and the amount of bound dye. Moreover the accuracy of the dye-binding method seems sufficient to be put in practice in meat plants.

Now we are trying to accelerate the dye-binding method by simplifying and mechanizing several steps.

2. METHODS

2.1. Samples

We analyzed different muscles or groups of musoles of veal, pork and beef from several carcasses.

2.2. Determination of the protein content

The nitrogen content was determinated in duplicate by the Kjeldahl method. We used HgO as a catalyst and a Parnas-Wagner apparatus for the steam distillation of the ammonia.

The protein content was calculated by multiplying the found nitrogen content by 6.25.

2.3. Determination of the amount of bound dye

The method used was described recently by Meester and Krol (4). The amount of bound dye was expressed as mg dye (Cochineal red A) per gramme of meat.

As the protein content of most of the samples fell into a narrow range, it was not possible to calculate the regression equations sufficiently exact. Therefore we used a modification of this method. Besides, taking exactly 2.0 g of meat in duplicate, we also took duplicates of exactly 1.8 and 2.2 g or 1.8, 1.9, 2.1 and 2.2. g of meat for the determination of the amount of bound dye, thus obtaining 6 or 10 replicates. In this way we got results representing a 10 % (or 5 %) lower or higher protein content than the samples and we could calculate the regression equations more exactly.

2.4. Calculations

We calculated the following characteristics:

- the standard deviation of the protein content, determined according to Kjeldahl;
- the standard deviation of the amount of bound dye;
- the regression equations, indicating the relation between the protein content (x %) and the amount of bound dye (y mg/g meat);
- the correlation coefficient between the protein content according to Kjeldahl and the amount of bound dye;
- the standard deviation of the regression, indicating the difference between the protein content according to Kjeldahl and the protein content calculated from the amount of bound dye by means of the regression equation.

3. RESULTS

3.1. Accuracy of the methods

We calculated from 130 duplicate determinations of the protein content of meat, according to Kjeldahl, the mean value and the standard deviation of the protein content. This amounted to approximately 20 % ± 0.1 %. The relative value of the standard deviation was 0.5 %.

From about 500 duplicate determinations of the amount of bound dye it was calculated that the mean value and the standard deviation was approximately 75 mg ± 1.7 mg Cochineal red A/g meat. The relative value of the standard deviation was 2.25 %.

The amount of bound dye was determined by measuring the extinction of the dye solution. The relative value of the standard deviation of the extinction was 0.9 %.

3.2. Regression formulae

When we carried out six to ten replicate determinations in one sample, varying the weight amounts of meat from 1.8 g to 2.2 g, the formulae of the "sample" regression lines were calculated. The results are not mentioned here.

When samples of the same muscle or the same group of muscles of four or more carcasses were analyzed, the formulae of the "muscle" regression lines were calculated from the obtained data. In these cases the correlation coefficients were also calculated. The formulae of these lines, as well as the correlation coefficients, are put together in table 1.

When the protein content and the dye binding capacity of several muscles or groups of muscles of one carcass were determined, the formula of the "individual" regression line and the corresponding correlation coefficient were calculated from all data referring to this carcass. The individual regression formulae were calculated for eight carcasses as mentioned in table 2.

Finally, from comparable data obtained from one kind of meat, either from beef, veal or pork, the formulae of some "total" regression lines together with the corresponding correlation coefficients were calculated. The formulae of these lines are reported in table 3.

3.3. The deviation from regression

For every regression line, the standard deviation from regression was calculated. When a number of corresponding regression lines refers to the same group of determinations (the same kind of meat and the same number of replicates) the means of these standard deviations from regression were calculated. These mean values are reported in table 4.

4. DISCUSSION

Comparing the two methods for the determination of the protein content, it appears that the Kjeldahl-method is more exact than the dye binding method by four to five times. The relative values of the standard deviations were respectively 0.5 % and 2.25 %. Perhaps it is possible to reduce the difference in exactness between the two methods by a more accurate procedure of the dye binding method.

The regression lines form together a rather narrow sheaf, as appears from the small differences between the regression coefficients. Only in one case this agreement was less good. The individual regression lines showed a more pronounced divergence. This was mainly caused by the fact that the protein contents of the samples of lean meat of one carcass were only slightly different. To meet this inconvenience the range of the protein amount was enlarged by weighing not only 2.0 g of meat, but also 1.8 and 2.2 g or 1.8, 1.9, 2.1 and 2.2 g. In these cases the solution by wich the dye was bound contained up to 10 % more or 10 % less protein than otherwise.

The total regression lines of beef, veal and pork coincide closely. So, in our opinion, it is allowed to use in all cases the same regression line for these three kinds of meat. Only rind shows a deviating behaviour; perhaps this is also the case with other meat samples, containing a large amount of connective tissue. This was not investigated, all samples analyzed during our investigation being trimmed of fat and of coarse connective tissue.

The most important results of the calculations are the standard deviations from regression, or in other words the difference between the protein content determined according to Kjeldahl and the protein content calculated from the amount of bound dye using the corresponding regression formula.

Starting with the "sample" regression line, it appears that the relative value of the standard deviation from regression was on the average 2.0 %. This is nearly the same relative value as the standard deviation of the dye binding method, viz. 2.25 % (see § 3.1.).

Using the three other types of regression lines, the "muscle", "individual" and "total" regression lines, it appears that the standard deviations from regression were nearly the same. The relative values varied from 3.0 % to 5.0 %, mean value: 4.25 %, or expressing it in absolute data: about 20 % protein $\pm$ 0.85 % protein.

Therefore it makes no difference whether we use the "muscle", "individual" or "total" regression line for calculating the protein content.

This simplifies strongly the calculations, because we can only use now one regression formula to calculate the protein content from the amount of bound dye. By using a colorimeter with a special scale, divided in percentages of protein instead of the absorbance, it would even be possible to avoid the calculations.

Now we get advantage of the dye binding method: the quickness. All conditions are fulfilled to execute the determination quickly and even semi-automatically. This is the next research-object of our institute in this field.

Only one question remains. Does the greater quickness of the dye binding method counterbalance the lower exactness of the method, compared with the Kjeldahl method. We can answer this question in the affirmative, as it is not possible to use the Kjeldahl method for a quick check during the production of meat products. Thus we cannot get the full benefit of the higher exactness of this method. On the other hand, however, it is possible to draw profit from the advantage of the greater quickness of the dye binding method, because the exactness of the method ($\pm$ 5 % relative) is sufficient for supervision during the manufacturing of meat products.

SUMMARY

The protein content and the binding capacity for Cochineal red A were determined in 872 samples of meat. Several regression formulae were calculated for the relation between the protein content and the amount of bound dye.

It appears that the regression formulae for beef, veal and pork were nearly the same. Using these regression formulae, the protein content can be calculated from the amount of bound dye with a relative exactness of 5 %.

It is stated that this is sufficient for a rapid check of the protein content during the manufacturing of meat products.

ZUSAMMENFASSUNG

Der Eiweissgehalt und das Bindungsvermögen für Cochenillerot A sind in 872 Fleischproben bestimmt worden. Einige Regressionsgleichungen wurden für die Beziehung zwischen der gebundenen Cochenillerotmenge und dem Eiweissgehalt ermittelt. Es zeigt sich dass die Gleichungen für Rind-, Kalb- und Schweinefleisch nahezu gleich waren.

Mit Hilfe dieser Regressionsgleichungen ist es möglich den Eiweissgehalt aus der gebundenen Farbstoffmenge mit einer relativen Genauigkeit von 5 % zu berechnen.

Diese Genauigkeit erscheint ausreichend für eine schnelle Kontrolle des Eiweissgehaltes während der Fleischwarenherstellung.

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REMARKS

It will be possible to show during the meeting the following slides:

1. "Muscle" regression lines of veal
2. "Muscle" regression lines of pork
3. "Individual" regression lines of beef
4. "Total" regression lines of veal, pork and beef.

Table 1. "Muscle" regression formulae, indicating the relation between the protein content (x %) and the amount of bound dye (y mg/g meat), of a number of muscles or groups of muscles

kind of meat	muscle or muscle group	number of samples	regression formulae x = a + by a	b	correlation coefficient
veal	M. semimembranaceus	4[1)]	1.5	0.273	0.94
	M. ilio psoas	5[1)]	3.2	0.246	0.86
	M. adductores	4[1)]	1.3	0.274	0.93
	M. supraspinam	4[1)]	1.9	0.271	0.91
	M. triceps brachii	4[1)]	-1.8	0.329	0.93
	M. long. dorsi	4[1)]	1.7	0.264	0.91
pork	M. psoas	47	0.6	0.277	0.86
beef	shoulder	9	0.4	0.265	0.86
	M. long. dorsi	14	3.2	0.235	0.91
	fillet	12	1.3	0.245	0.85
	ribs	7	2.8	0.242	0.89
	thin flank	13	6.1	0.195	0.69
	sticking	8	2.9	0.232	0.92
	brisket	13	5.4	0.201	0.84
	neck (backside)	9	3.4	0.226	0.93
	shin	14	5.1	0.208	0.85
	M. semimembranaceus	13	1.3	0.252	0.93
	M.quadriceps femoris	11	-2.9	0.304	0.98
	silverside	13	5.1	0.207	0.77
	rump	13	1.3	0.258	0.88
	thick flank	7	3.2	0.223	0.84
	M. adductores	9	1.5	0.249	0.94

1) Each sample was analyzed in 10 replicates

Table 2. "Individual" regression formulae, indicating the relation between the protein content (x %) and the amount of bound dye (y mg/g meat), of a number of carcasses

carcass number	kind of meat	number of samples	number of determinations	regression formulae x = a + by a	b	correlation coefficient
1	beef	6	12[1]	13.4	0.101	0.65
2		11	22[1]	12.8	0.105	0.32
3		14	28[1]	14.3	0.091	0.34
4		11	22[1]	7.5	0.179	0.79
5		11	22[1]	-21.5	0.517	0.84
6		15[2]	142	2.3	0.246	0.89
7		15[2]	147	3.4	0.227	0.85
8		12[2]	72	0.2	0.263	0.95

1) Regression formulae calculated from the means of duplicate determinations.

2) From each sample were 6-10 replicates analyzed.

Table 3. "Total" regression formulae for a number of series of beef, veal and pork samples, indicating the relation between the protein content (x %) and the amount of bound dye (y mg/g meat)

kind of meat	number of samples	number of determinations	regression formulae x = a + by a	b	correlation coefficient
veal	25	229[1]	1.9	0.267	0.90
pork	28	56[2]	0.2	0.272	0.98
	4	24[3]	3.4	0.222	0.87
	75	150[2]	-0.7	0.291	0.98
beef	183	183[4]	5.0	0.207	0.80
	42	361[1]	2.0	0.247	0.88

1) From each sample were 9 or 10 replicates analyzed

2) From each sample were duplicates analyzed

3) From each sample were 6 replicates analyzed

4) The regression formula is calculated from the means of duplicate determinations

Table 4. Mean standard deviations from regression, calculated from corresponding determinations. (Mean protein content: about 20 %).

regression line	kind of meat	number of regression lines	mean standard deviation % protein	mean relative value
"sample"	veal	25	± 0.40	
	pork	5	± 0.41	2 %
	beef	42	± 0.40	
"muscle" (table 1)	veal	6	± 0.60	
	pork	1	± 0.51	4.3 %
	beef	15	± 0.96	
"individual" (table 2)	beef	3	± 0.77	4.4 %
	beef	5	± 0.96	
"total" (table 3)	veal	1	± 0.65	
	pork	1	± 0.76	
	pork	1	± 0.78	4.0 %
	beef	1	± 0.82	
	beef	1	± 1.00	