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Rapid determination of protein in meat and meat products

by

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1. INTRODUCTION

For the determination of the total protein content in meat and meat products the Kjeldahl method is generally used. As early as 1883 this method was developed and as a standard method it is excellent but very time-consuming and therefore less suitable for application in the analytic production control of the meat industry.

Investigations have been made if the dye binding method, on a large scale applied in dairy laboratories in particular, should do better for this purpose. This method was first applied by Udy (1-2) for the determination of the protein content in wheat flour, and since that time it was developed by many investigators (3-8) for milk especially. Freimuth (9) applied the dye binding method to meat and sausage.

The principle of the method is very simple. A solution or suspension of a protein is mixed at a definite pH with an excess of dye solution. Part of the dye is bound by the protein present, after which the insoluble dye-protein complex thus formed is removed by centrifugation. The concentration of the remaining dye is then a measure for the protein content of the original solution. Previously a determination can be made on the relation between the amount of dye bound and the protein content determined according to Kjeldahl.

2. METHOD

2.1. General

The proteins of the samples of meat or meat products are to be brought into solution or into an utterly fine state of suspension in order to enable them to react quantitatively with the dye solution.

As grinding of the sample with the aid of a waring blender, a colloid mill or ultrasonic vibrations did not give satisfactory results because of the often occurring coagulation, a high speed mixer of the "Ultra-Turrax" type was used for this purpose. Thus the samples can be made into a 'meat-milk' in a few seconds.

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When in doing so the samples are not ground in water, but in formic acid, the proteins appear to dissolve for the greater part, whereas in addition the fat present is suspended well. In order to save time in applying this method, previous defatting of the samples was foregone.

The "semi-solutions" obtained from the samples in this way can be easily pipetted and diluted with formic acid.

Following Freimuth (9), cochineal red A (= ponceau 4 R) was chosen as dye. A solution of amidoblack10 B, as is mostly used for protein determinations in milk, gave equally good results, but offers greater difficulty in visual check up of turbidities.

The method was applied on beef and pork as well as on samples of sterilized luncheon meat.

2.2. Apparatus

Laboratory-size meat mincer, fitted with a 3-mm plate.

Ultra-Turrax high speed mixer, type T.P. 18/2, made by Janke & Kunkel KG., Staufen i. Br. (Germany).

Laboratory centrifuge, capacity of tubes about 15 ml.

Colorimeter, e.g. Engel-colorimeter, made by Kipp, Delft (The Netherlands). Filter used: no. 53, about 530 mμ.

2.3. Reagents

Formic acid, a.r., 98-100 %.

Buffersolution of pH 2.2: 20.7 g of citric acid monohydrate, 1.44 g of $Na_2HPO_4 \cdot 2$ aq and 0.5 ml of 10 % alcoholic thymol solution, diluted with water to 1 l.

Cochineal red A (C.I. 16255; Schultz no. 213) 1 mg per ml buffersolution of pH 2.2.

Carbon tetrachloride.

2.4. Procedure

2.0 g of the sample, after having been minced twice in a meat mincer, are ground for 30 seconds together with about 15 ml of formic acid by means of an Ultra-Turrax high speed mixer. With the help of formic acid the mixture is quantitatively transferred into a 25 ml volumetric flask and filled up to the mark with formic acid.

After mixing, 1 ml of the resulting suspension is pipetted into a centrifuge tube and 0.5 ml of carbon tetrachloride is added; this mixture is shaken vigorously for a short time in order to dissolve the fat.

Then 10 ml of cochineal red solution are pipetted into the tube and the mixture is thoroughly shaken and mixed for 30 seconds. (Note that with some kinds of products a better defatting is obtained by adding the carbon tetrachloride after the addition of the dye).

After 5 minutes' centrifugation at 3000 r.p.m., 1 ml of the clear supernatant dye solution is diluted with buffersolution up to 50 ml, after which the extinction E_c of this solution is colorimetrically determined in a cell of 1 cm pathway at 530 mμ, with respect to the buffersolution. The extinction E_o of the original dye solution is determined in the same manner.

2.5. Calculation

The amount of cochineal red in mg bound per gramme of the sample is:

$$\left[V.c_o - (V + v).c_o.\frac{E_c}{E_o} \right] . \frac{V_o}{v.G}$$

in which V = millilitres of added dye solution;
c_o = concentration of original dye solution in mg per ml;
v = millilitres of sample suspension used;
G = grammes of sample, dissolved in V_o ml of formic acid;
E_o = extinction of original dye solution;
E_c = extinction of dye solution after centrifugation.

According to the directions given under 2.4. this consequently comes to:

$$\text{mg of dye bound per g of sample} = (10 - 11 . \frac{E_c}{E_o}) . \frac{25}{2}$$

Standard curves can be made by graphing the obtained values against the protein contents found according to the Kjeldahl method. In order to save figure-work, the value of E_c/E_o can of course also be used for it.

3. RESULTS AND DISCUSSION

3.1. Cochineal red solution

Dilutions of a cochineal red solution (concentration 1 mg per ml of buffer) were made in buffersolution, and their extinctions determined. From the results (see fig. 1) it appears that the cochineal red used by us (made by Polak & Schwarz, Red W.W. 509624) does not fully conform to the law of Lambert and Beer. The deviation from the straight line, however, is only slight and was left out of the calculations.

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3.2. Reaction time

Suspensions of luncheon meat samples were shaken for 30 seconds or stirred for 5 minutes respectively with the dye solution. From the extinction values obtained after centrifugation it appeared that 30 seconds' shaking is sufficient for complete formation of the complex; prolonged mixing does not result in lower extinction.

3.3. Reproducibility

The reproducibility of the controls was determined by calculating the standard deviation of duplicate determinations.

The standard deviation of the extinctions measured in 31 duplicate determinations of sterilized samples of luncheon meat appeared to be: $s = 0.0158$. So the maximum relative error amounts to about $3s = 0.047$, which means about 5 % in the duplicates.

3.4. Dye binding capacity

It is a well-known fact that the dye binding capacity of proteins at a certain pH is not constant, but depends on the concentration of both protein and dyestuff.

As for meat this dependence was ascertained according to the method described in 2.4., first by causing meat suspensions of varying concentration to react with the same cochineal red solution, and on the other hand by causing increasing concentrations of dye solution to act on one and the same meat suspension. The results are given in figs 2 and 3.

With increasing protein concentration (fig. 2) the extinction appears to decrease practically linearly to a minimum, whereat practically all dye is bound. In further increasing the protein concentration, however, less dye is bound; maximum binding occurs at about 80 mg of meat per ml.

From fig. 3 it appears that with increasing dye concentration there is a strong rise of the dye binding capacity of the protein, until a maximum value is reached. With higher dye concentrations a slight decrease of the binding occurs.

These facts can be accounted for by stating that after adding dye anions to the protein, a soluble dissociable complex is formed. On increasing the quantity of dyestuff (fig. 3), at a definite ratio between dye and protein all the cationic reactive centres of the protein molecules will have been occupied by dye anions and the complex, insoluble now, will precipitate (isoelectric point). Then further

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increasing the dye concentration only results in an increase of the amount of non-bound dye in the reaction mixture, which means an increase of E_c/E_o. When, on the contrary, at constant dye concentration, the protein concentration is increased (fig. 2), initially all of the protein will be precipitated as a complex (as there is an excess of dye), until, arriving above the protein dye ratio mentioned before, there is too little dyestuff available to neutralize all the positive charges of the protein molecules; then a soluble complex arises again, the extinction increases and the amount of dye bound per protein unit falls.

According to Russian investigators (10-11) the reactive centres of the protein molecules consist of a combination of cationic and nonpolar groups (the latter attainable for the apolar groups of the dye anions). Moreover, they are assumed to exist in two types, one occurring in a small number on the surface of the protein molecules and with a strong affinity to polyvalent dye anions, and the other type, in a greater number, with a weaker affinity to univalent anions. At low protein concentration and a large excess of dye this dye is supposed to be taken up by the second type, whereas the dye, as soon as there is an excess of protein, should preferably be bound by the first type of reactive centre.

3.5. Relation between Kjeldahl-determination and dye binding

As shown already under 3.4. the dye binding method is rather sensitive to slight variations in the dye concentration. Besides, there is no linear correlation between the dye binding and the protein contents as determined by the Kjeldahl method; the deviation from linearity, however, will be small, because in practice the protein concentration in meat products varies only between fairly narrow limits. This is demonstrated by the results of a series of sterilized samples of luncheon meat with different composition, of which the relation between dye binding and Kjeldahl findings is given in fig. 4. As is to be seen here, these standard curves are quite useful.

3.6. Some factors influencing the dye binding method

As the dye binding capacity depends on the nature of the proteins used, meat products in which varying proportions of the various carcass parts have been used, will show a different dye binding in respect of the same Kjeldahl protein content. The manner of pretreatment of the meat constituents that are to be processed is also likely to play a part. These items will also have to be investigated,

in doing which in particular the dye binding capacity of the carcass parts should be determined.

As the protein determination according to the dye binding method is principally different from the Kjeldahl method, further attention will have to be given to the influence of ammonia, free amino acids and peptides on the results of the determination.

Another cause of errors might be found in the different degrees of heating to which the meat products have been exposed, i.e. in the change of dye binding capacity of the meat proteins by heat denaturation.

To control this, some samples of beef were heated in a tube placed in boiling water during 30 minutes. The dye binding reaction was performed on these samples, both before and after the heating. From the resulting extinctions (see table 1) it appears that heat denaturation has little or no effect on the cochineal red binding at pH 2.2. This is in accordance with the investigations by Hamm and Deatherage (12), who found that heat denaturation of beef delivered no significant change in the number of basic groups of muscle proteins.

Table 1. Effect of heating on dye binding of beef.
Extinction E_c before and after heating

	Before heating	After heating
Sample 1	0.183	0.191
Sample 2	0.191	0.191
Sample 3	0.273	0.267

As in Germany milk protein and in the United States milk protein and soya preparations are allowed in meat products, and these proteins as a rule possess a dye binding capacity different from that of meat protein, it seemed useful to us also to investigate a possible interfering influence of these kinds of protein.

From fig. 5 (based on the data of fig. 4) we get a picture of the quantity of cochineal red bound per gramme of meat protein, for a series of sterilized luncheon meat samples in which no foreign proteins had been processed. This quantity, as could be expected, is not constant, but depends on the protein concentration; the average value is 276 mg per gramme of meat protein.

For comparative purposes we defined the relation between dye binding and protein concentration of a caseinate allowed in meat products in Germany (a trade product with a protein content of 83.9% calculated with the factor 6.25), by means of a series of solutions in formic acid. The preparation was not sterilized in advance. From fig. 6 it is seen that this protein has another concentration colour ratio than meat protein; a maximum is reached already at a low concentration of the protein. The average value of 273 mg cochineal red bound per gramme of caseinate protein, however, corresponds with the one given by meat.

Of soy protein preparations, too, the colour-binding capacity can be expected to be quite different from the one shown by meat. For one of these trade products for instance,(an U.S. product with a protein content of 52.3 %, calculated with the factor 6.25), the binding appeared to be considerably higher, viz. an average of 329 mg of cochineal red per gram of soy protein.

From these results it readily appears that these foreign proteins, when used in unknown quantities in the meat products, will be a source of error for the determination.

From the dye binding results of series of luncheon meat samples in which 2 % caseinate or soy preparation respectively were processed, as compared with those of the normal series without foreign proteins as described earlier, it is to be seen (fig. 7) that it is surely possible to set up reasonable standard curves if these proteins have been processed in the meat products in constant known percentages.

4. CONCLUSION

The dye binding method as discussed in the preceding presents great possibilities as a useful and rapid procedure for the determination of the protein content of meat and meat products. The method described is rather primitive as yet, but can no doubt be improved technically and possibly be mechanized, as has been done in dairy laboratories.

Although it will probably not be possible to apply this method to meat products of unknown formula in control laboratories in substitution for the Kjeldahl determination, it may be very valuable for the laboratories of the meat industries themselves, where, with the formulas known there to work upon, standard curves can be drawn up empirically for the finished products.

By means of the dye binding method it will be possible, too, to collect a great number of data in a rapid and simple way, regarding the protein contents of the types of meat and the parts of the carcass to be processed, required for a reliable calculation of costings and formulas.

SUMMARY

The dye binding method by means of cochineal red A for a rapid determination of the protein content was made practicable to be applied to meat and meat products. Reaction conditions and interfering factors were investigated more closely. The results obtained by this method appeared to correspond well with those according to Kjeldahl.

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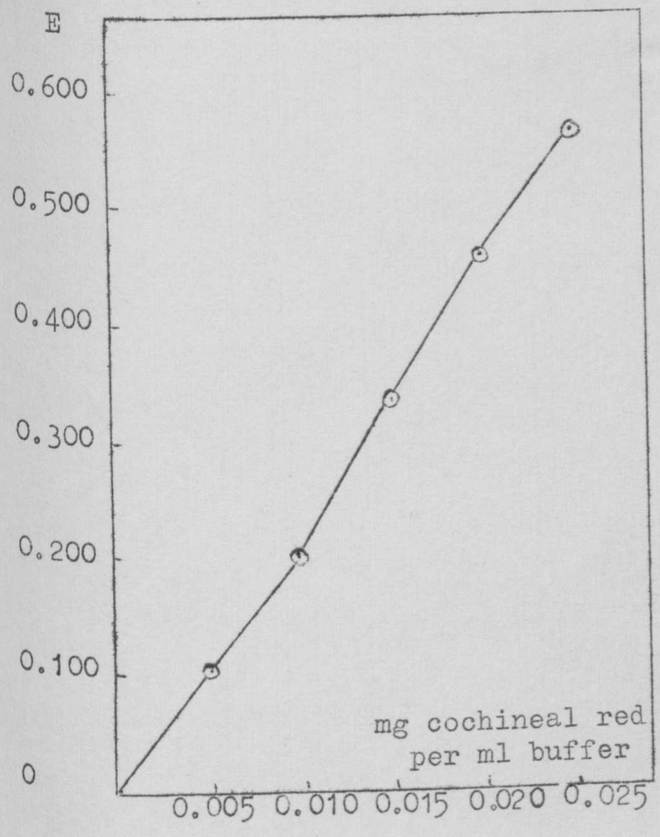

Figure 1. Extinction of cochineal red in buffersolution

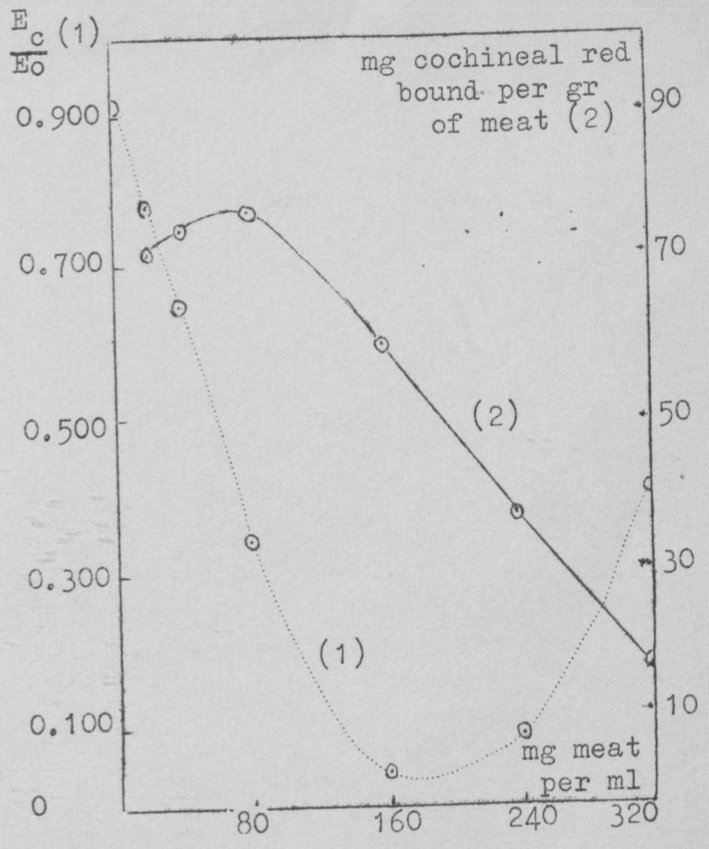

Figure 2. Binding of cochineal red by increasing meat concentrations
Dye conc. 1 mg per ml
$E_o = 0.574$

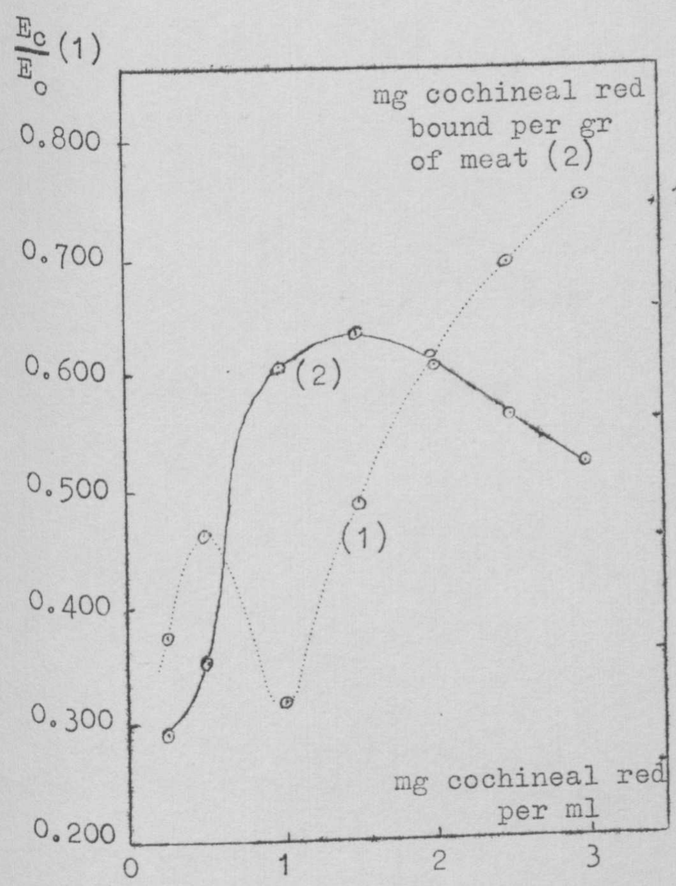

Figure 3. Binding of cochineal red by meat with increasing dye concentrations
Meat conc. 80 mg per ml

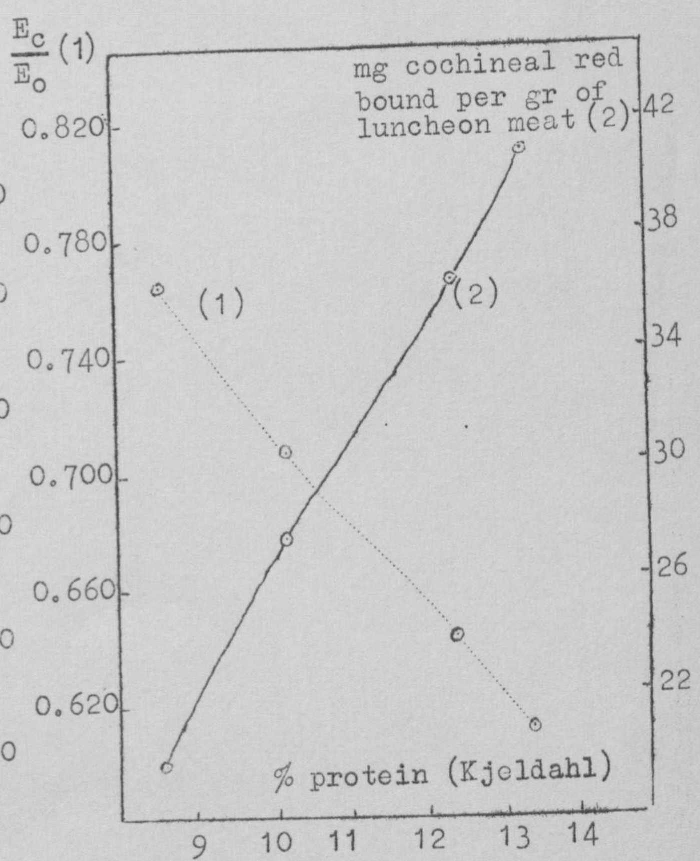

Figure 4. Binding of cochineal red by luncheon meat

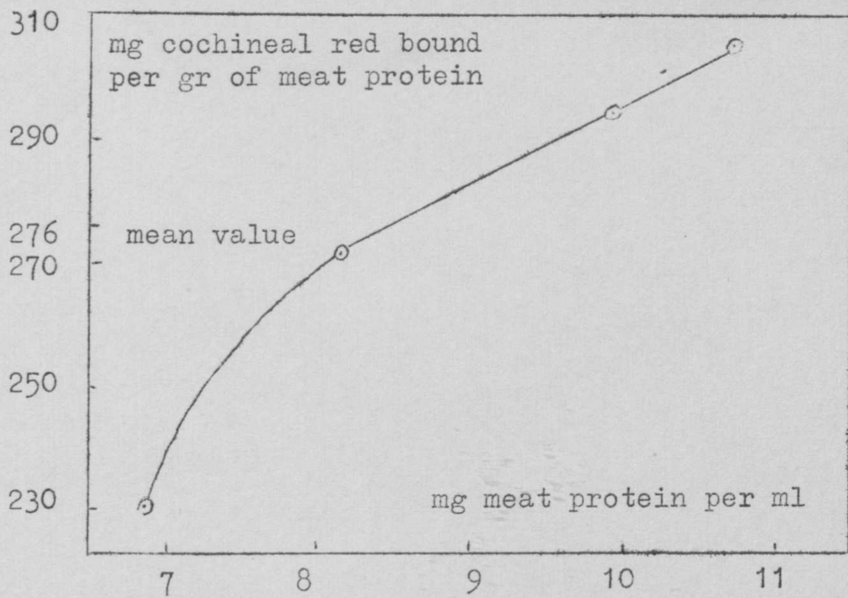

Figure 5. Binding of cochineal red by luncheon meat

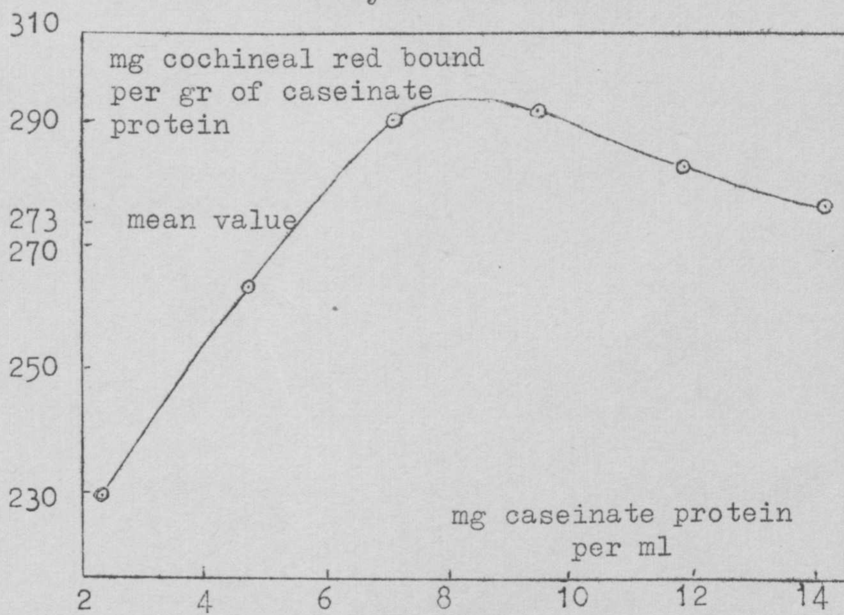

Figure 6. Binding of cochineal red by caseinate solutions

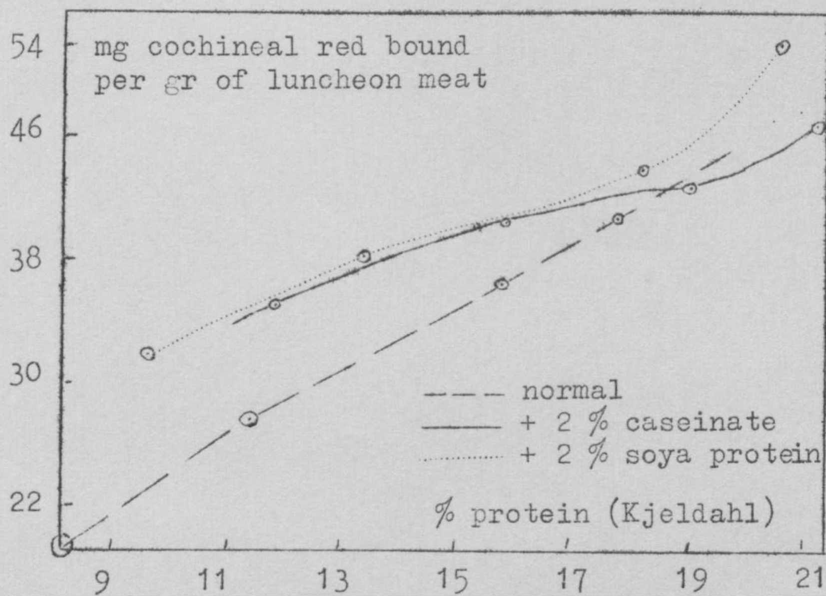

Figure 7. Binding of cochineal red by luncheon meat containing milk and soya protein