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The detection of soya bean protein in meat products

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Introduction

With increasing use being made of proteins from sources other than meat, such as soya bean, it is desirable to have a method of identifying these proteins in meat products. Olsman[1] described a procedure using starch gel electrophoresis in urea by which soya protein was detected. Such a method separates proteins according to their charge but some areas in the electrophoresis pattern have no distinguishing features when mixtures of proteins are being examined. Electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulphate separates proteins according to their molecular weight and clearer results have been obtained from mixtures of muscle proteins. The proteins of the myofibril have been successfully separated and identified by this method [3] and the proteins of soya bean have been shown in a preliminary paper[4] to be sufficiently different from those of meat to identify the presence of soya protein in meat.

Experimental

Preparation of samples

Meat, either raw or cooked to 100°, was minced and treated with several volumes of acetone to remove the fat and moisture. The dried product was ground to a fine powder. Textured soya bean protein or soya flour was used untreated. Commercial sausages (British Manufacture) which were known to contain added soya flour were treated as meat.

To prepare samples for electrophoresis weighed amounts of the dried meat powder and soya bean were mixed in different proportions and suspended in 10 ml of 3% sodium dodecyl sulphate containing about 0.5% mercaptoethanol. The suspensions were heated in a boiling water bath for 30 min. to dissolve the proteins. While still hot the samples were centrifuged at 30,000 g for 30 min and then filtered to remove any floating material.

Gel Electrophoresis

Electrophoresis was carried out on a slab of polyacrylamide by the procedure described elsewhere[3]. The acrylamide concentration was 8% with 1:30 (w/w) crosslinker in a buffer of 30 mM boric acid, 30 mM Tris with 0.1% sodium dodecyl sulphate and 0.1% mercaptoethanol. The buffer in the electrode trays was saturated sodium tetraborate with 0.5% sodium dodecyl sulphate.. The apparatus was water cooled to keep the temperature between 20° and 30° with a voltage of 180 volts applied across the gel to give a current of 50-60 m amps. After 4 h. the gel was sliced to remove the top and bottom layers of the slab and the middle slice was placed in a wash of 30% methylated spirits and 5% acetic acid in water. After 2 h. the gel was stained overnight in 0.2% Coomassie brilliant blue. Repeated washings in the methylated spirits and acetic acid mixture were made until the gel had cleared.

Gel Scanning

Strips for each sample run, were cut from the gel and scanned in a Unicam SP 500 spectrophotometer at 560 mμ, using a specially constructed attachment consisting of a clear perspex holder (to take gel strips) fitted to a plate which was free to rotate in the cell compartment of the spectrophotometer. The plate was rotated by an external motor giving 4 revs./hr. so that the complete gel strip was scanned in 4 min.

Results

The gel patterns of the soya bean protein and the meat proteins were completely different. The pattern found for meat proteins (Fig. 1a) was typical of the pattern of myofibrils[3] showing bands characteristic of myosin, actin, tropomyosin, troponin and α-actinin. The sarcoplasmic proteins no doubt also contributed to the gel pattern but individual proteins were not

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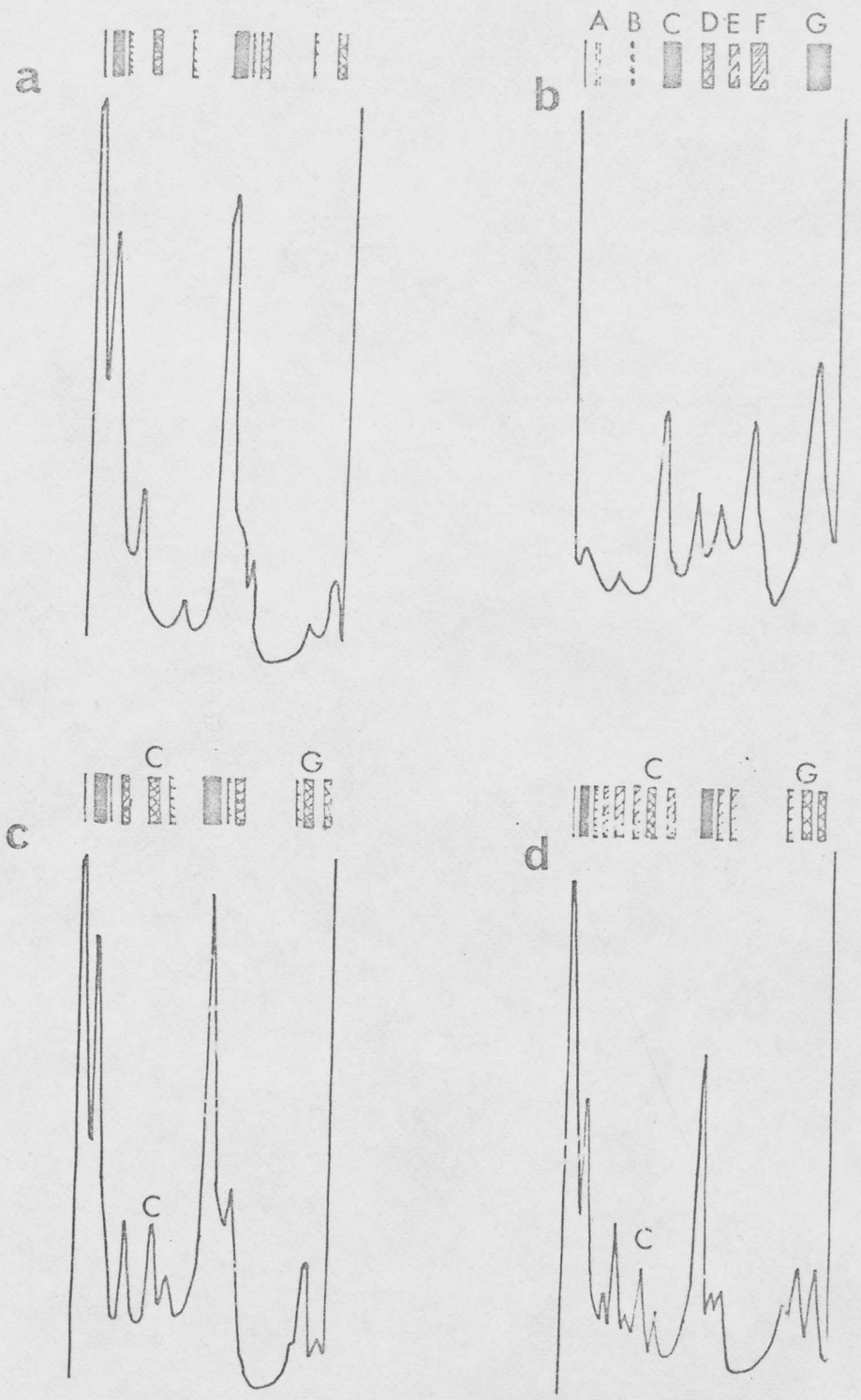

Fig. 1. Facsimiles of the polyacrylamide gel patterns of meat and soya proteins with the corresponding trace obtained by scanning the gel at 560 mμ

a) 50 mg of dried cooked meat powder
b) 50 mg of soya powder
c) 50 mg of dried meat powder with 30 mg of soya powder
d) 100 mg of dried raw sausage mix

All quantities were dissolved in 10 ml of 3% sodium dodecyl sulphate

identified. Of the soya proteins (Fig. 1b) two bands C and G, were much stronger than the others and did not coincide with any of the bands from meat protein. The appearance of these two bands in a gel pattern, therefore, is good evidence of the presence of soya protein in the sample. Fig. 1c was a mixture of soya bean powder and meat and Fig. 1d was a commercial sausage of British type and manufacture. Both these samples showed the C and G bands of soya protein quite distinct from the meat proteins but the other soya bands as expected merged with the meat protein bands. The sausage also showed bands, not found in the meat protein pattern, the source of these is not known but they do not interfere with the identification of soya protein.

The patterns obtained by scanning the gels areshown in Fig. 1 underneath the individual gel pattern. It was found that the G band of soya which was the most intense band was too close to the troponin bands of meat to give a definite peak (Figs. 1c and 1d). The C band however gave a clear peak with no interference from any meat bands and therefore was chosen to make a quantitative assessment of the amount of soya protein mixed with meat.

Mixtures containing different proportions of soya powder and dried meat powder up to a total of 100 mg were extracted with 10 ml of 3% sodium dodecyl sulphate. The gel patterns obtained on electrophoresis were similar to Fig. 1c. with the intensity of the C band increasing as the proportion of soya powder increased. Analysis of the peaks obtained by scanning the gel strips showed that size of the peaks (as measured by the weight of the cut out peak) in duplicated samples was not identical due mainly to variations in slot size, gel thickness, and degree of staining. But the ratio of the weight of the C peak to the weight of the actin peak in the same gel strip gave much more consistent results.

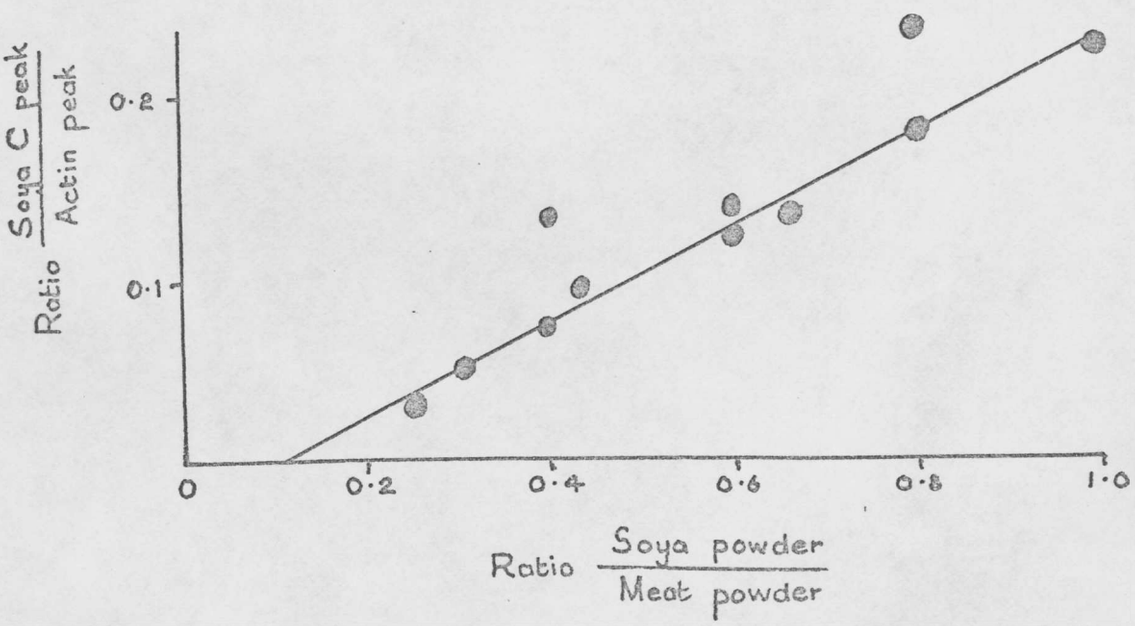

Fig. 2 shows the results of the plot of the ratio of the C peak to the actin peak against the ratio of dried soya powder to dry meat powder. The best fit for a straight line does not pass through the origin. This could be due to the background stain present in every strip which tends to mask the C peak at low concentrations of soya powder.

To test the reliability of Fig. 2 as a standard curve for the estimation of the amount of soya powder added to a meat product, the results for the sausage mix, used in Fig. 1d were analysed. Because the results in Fig. 2 were on a dry weight basis it was necessary, first, to know the amount of dry meat protein in the wet sausage mix. From a moisture content of 45% and a fat content of 30% the amount of fat free dry matter in the sausage mix was 25%. The amount of dry meat protein in the dried sausage mix was determined by a comparison of the gel strips of sausage mix and meat protein which showed that the actin peak from 200 mg of dried sausage mix was equivalent to the actin peak of 50 mg of dried meat protein. Thus only 25% of the dried sausage mix was dry meat protein. Therefore the dried meat protein content of the wet sausage mix was 6.25%.

The ratio of the soya C peak to the actin peak was determined on five different portions of 200 mg of dried sausage mix and gave an average of 0.097 ± 0.007 which from Fig. 2 was equivalent to a ratio of soya powder to meat protein of 0.44. Therefore, on the basis of the calculation of dry meat protein content above, the amount of soya powder in the wet sausage mix was 0.44 x 6.25%, or 2.7% which was in good agreement with the 2.5% soya powder which the sausage manufacturers claimed they were adding.

Conclusion

There is no doubt that the addition of soya bean protein to meat products can be detected by the presence of the two main bands of soya protein in the pattern obtained by electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulphate. The limit of detection is about 5% soya bean powder in meat on a dry weight basis. The quantitative evaluation is only in the preliminary stages but the results from a small number of gels are encouraging and the method should offer a basis for assessing quantitatively the amount of soya protein added to meat.

References

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