DETECTION AND DETERMINATION OF SUNFLOWER AND SOYBEAN PROTEINS IN MEAT PRODUCTS

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INTRODUCTION

The increasing utilization of vegetable proteins in meat products or in foodstuffs resembling meat products has drawn attention to the necessity of developing suitable methods for the detection of vegetable proteins contents (**).

For this aim many methods have been extensively investigated and proposed for detection and determination of these meat extenders, expecially for soybean products. A very complete and elaborated survey on this subject is given by Olsman and Krol (1).

The published methods of analysis for soy proteins are based on various principles. Each has only limited applicability, depending on both the type of soy preparation concerned and the heat treatment o the products.

A division into two main groups can be made (2): A) Methods based on the assay by the chemical or microscopic procedures of substances accompanying the soybean proteins (satellite detection methods). B) Methods based on the recognition and measurement of the soybean proteins themselves by electrophoresis, immunochemistry, amino acid composition or sequence analysis, etc.

No such research effort, however, has been reported for the detection and quantitative assessment of other vegetable proteins, such as sunflower, one of the most promising new source of edible products. For sunflower products, just recently, electrophoretic and immunochemical methods have been partially investigated (3-5). Many difficulties

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(**) In our country (Italy), regulations (D.M. 4-8 +1979) restrict the quantity of soy protein meal, concentrate and texturized to maximum levels of 30%.

arise from crossed reactions with other vegetable proteins (rapeseed, field bean) and when sunflower proteins are submitted to heat treatment which destroys some antigenic properties and decreases the dye binding capacity of proteins. The technique of electrophoresis, under denaturing conditions, seems to be one of the most promising and accurate methods available today for quantitative assessment of protein content in meat products. We concentrated our efforts on finding a method with high precision for the quantitative analysis of sunflower/meat products.

RESULTS AND DISCUSSION

Typical SDS 1% polyacrylamide pherograms in 1% β-mercaptoethanol, obtained from sunflower/meat and soybean/ meat blends are shown in fig. 1, 2.

Laser densitometer trancing from electrophoretograms of a sunflower/beef and soybean/beef mixtures are shown in fig. 3, 4. The utilization of a slab gel system of increased length and of a laser scanning give satisfactory results with soybean-meat, sunflower-meat and sunflower/soybean/meat mixtures.

This arrangement allowed us to obtain an excellent resolution. It is possible to detect the vegetable proteins in raw and heated (up to 100°C) meat-soybean and meat-sunflower blends down to the levels of 2.5-3% of the total contents.

As you can see (fig. 2, 4), there are several soybean protein bands, in areas where there is little or no interference form meat proteins (namely R, S and T respectively). For calibration, the area under the soybean peaks was used, instead of that under peak T, as in the paper by Lee et al. (6), because it was more clearly resolved and not affected by some irregularities in intensity and mobility.

For meat and sunflower proteins we used the area under the actin (A) peak and under the G peak, respectively (fig. 3). The intensity of the actin band is very stable even at high temperatures ($>$ 100°C). The sunflower ban (G) interferes a little with meat proteins expecially at low sunflower contents; nevertheless it was possible to resolve this band and to detect a sunflower protein content of 2.5% in meat blends.

For the quantitative evaluation of sunflower and soybean content, in protein mixture with meat, a calibration curve (6, 8) was constructed using mixture of known composition.

When the ratios of the areas of component G (sunflower) and

component A (meat), in each mixture, were plotted against the relative composition of the mixture, a linear relationship was obtained (fig. 5). The same procedure was used for soybean/beef mixtures (fig. 6).

Regression coefficients were respectively: R_G 0.995 and R_S = 0.994. Heating the mixtures at 100°C for 20 min doesn't influence the intensity and electrophoretic mobility of the stained protein bands. At temperatures above 100°C, the intensity of the stained bands gradually decreases, making quantitative determination progressively difficult and indaginous. This decrease is only in part due to a decresging solubility of proteins but rather to probably alteration of functional groups involved in dye binding (9).

A three component system containing sunflower, soybean and beef proteins was also investigated to evaluate mutual effects among components (fig. 7).

The diagnostic bands of sunflower, soybean and meat proteins were still clearly separated from each other: optical density measurements were not interfered and quantitative determination could be carried out using the method previously described.

An additional study was conducted to evaluate the interference, between soybean and sunflower proteins, and various non-meat proteins, frequently added to meat products, e.g. casein, egg white, whey and wheat proteins. The electrophoretic patterns and relative mobilities of these meat additives (fig. 8) clearly indicate that there is no interference with the diagnostic bands of soybean, sunflower and beef proteins; they can be easily distinguished from one another.

The present electrophoretic procedure may be particularly useful in detection and quantitative determination of vegetable proteins such as sunflower and soy proteins in beef blends, because it directly identifies and quantitates the vegetables protein added. Like other methods, involving full denaturation of the protein by SDS, the present procedure minimizes the analytical complications caused by partial denaturation.

As reported by literature, we also encountered some difficulties in quantitative determination of protein products heated at temperatures above 100°C.

The experimental modifications, here described, increased resolution and sensitivity making it possible to measure the sunflower protein contents in meat blends, which cannot be easily carried out by traditional disk-gel electrophoresis.

EXPERIMENTAL

Fresh ground beef, commercial defatted soybean meal (Protamine 270 - CARGILL Amsterdam), sunflower concentrate (obtained in our bench-scale system and various blends (beef/soy and beef/sunflower)) in different preparations were prepared according to the experimental procedures described by Lee et al. (6). The different mixtures, were formulated to contain different amounts of vegetable proteins (from 2.5 to 50%) as indicated in CEC Report (7):

"The proportion of vegetable protein products does not exceed 30%, based on the computation of crude protein content (N x 6.25) for both the vegetable and the meat protein. The vegetable protein content is than express as

$$\frac{\text{vegetable protein (N x 6.25)}}{\text{vegetable protein + meat protein (N x 6.25)}} \times 100\%$$

samples were homogenized and extracted, for 20 min, in a boiling water bath, with a solution of 3% SDS, 1% B-mercaptoethanol in Tris-HCl buffer (pH 6.8).

The electrophoretic tecnique employed was a slight modification of that described by Lee (1975).

A vertical flatbed polyacrylamide gel slab of 1.5 cm width and 16-18 cm lenght instead of the original cylindrical gel columns was chosen because of its improved resolution and riproducibility. Dual vertical slab gel apparatus BIO-RAD, Model 221, was used for our purposes.

The relative concentrations of the stained protein components in the gels were measured by (transmission-type) laser densitometry at 633 nm. The peak areas of selected protein bands were measured by a HP (Hewlett Packard) 5840 A-GC integrating system.

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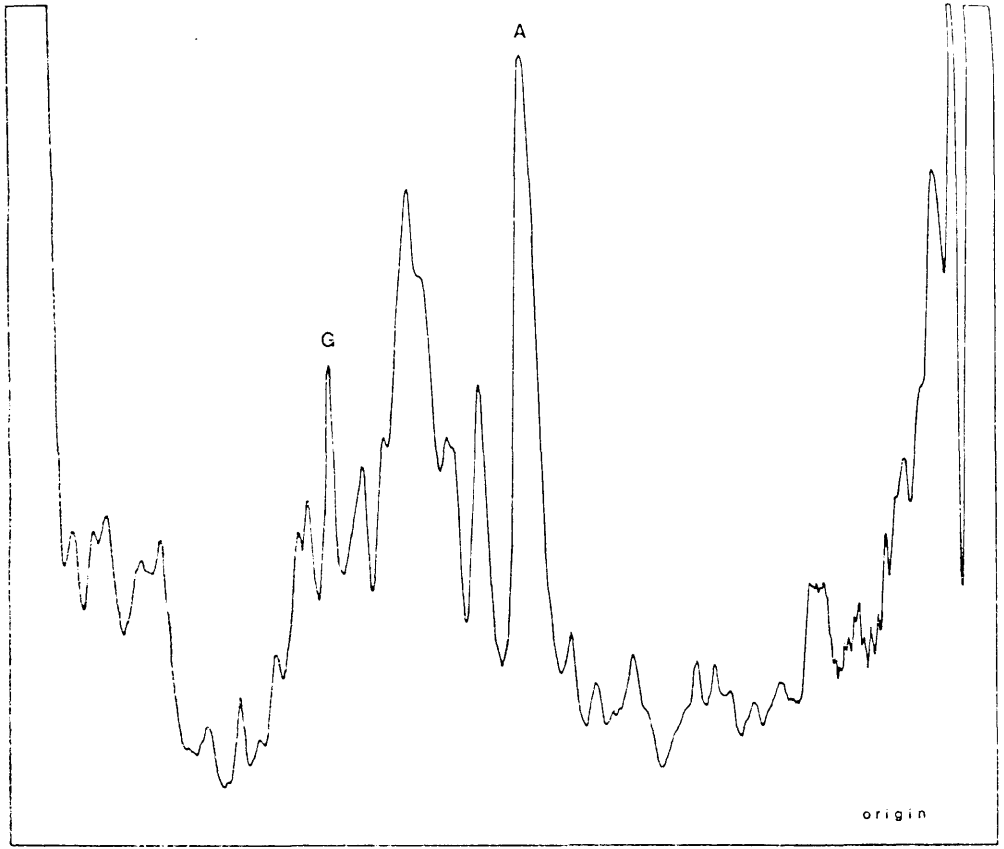
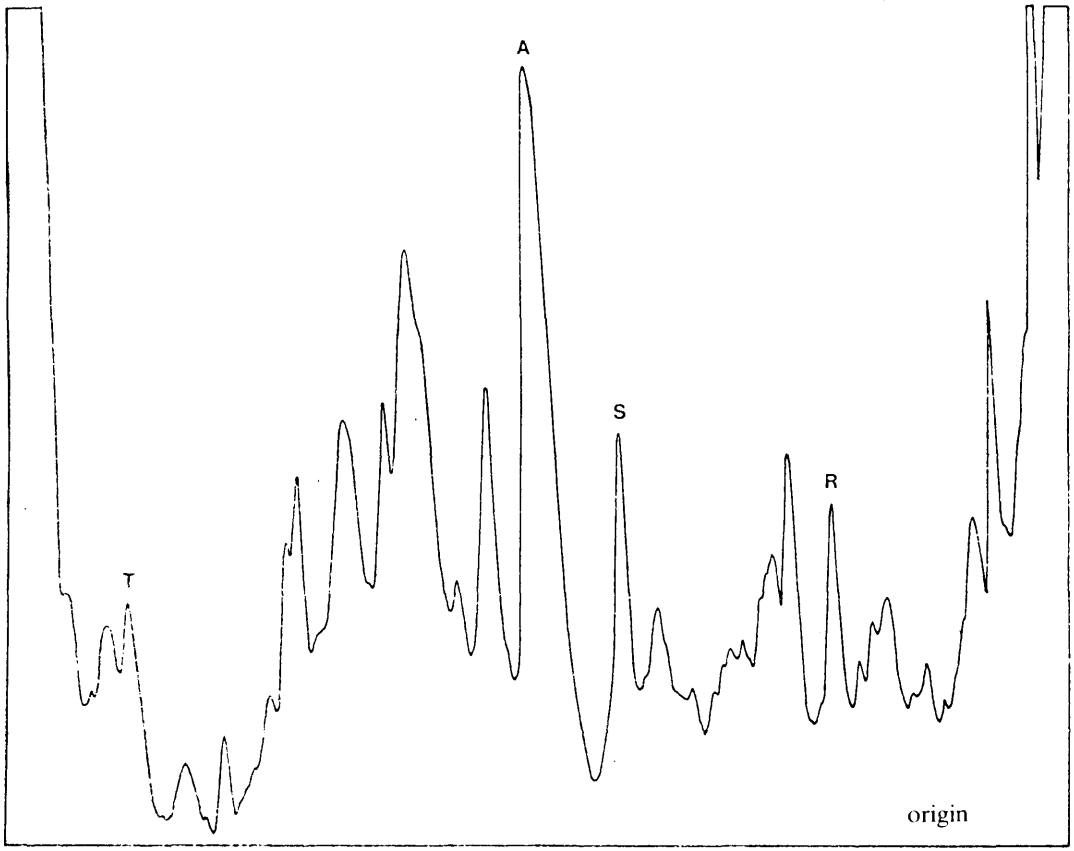


Densitometer tracing of polyacrylamide pherogram of sunflower-meat blend containing 30% of vegetable protein

A (actin) index band for meat protein

G index band for sunflower protein

Figura 3.



Densitometer tracing of polyacrylamide pherogram of soybean-meat blend containing 30% of vegetable protein

A (actin) index band for meat protein

S index band for soybean protein

R,T soybean protein band

Figura 4.

Relationship between the % composition of sunflower - meat mixtures and the ratio of the areas of components G and A

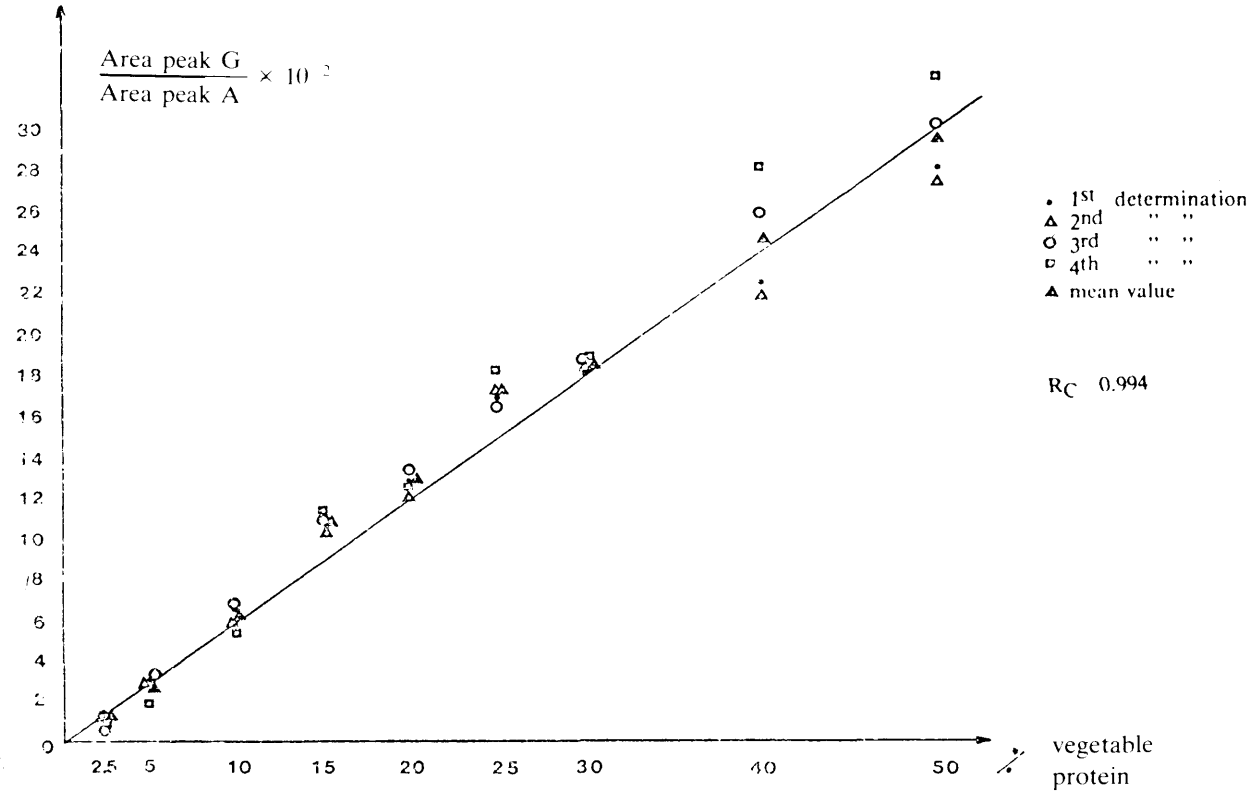


Figura 5.

Relationship between the % composition of soybean meat mixtures and the ratio of the areas of components S and A.

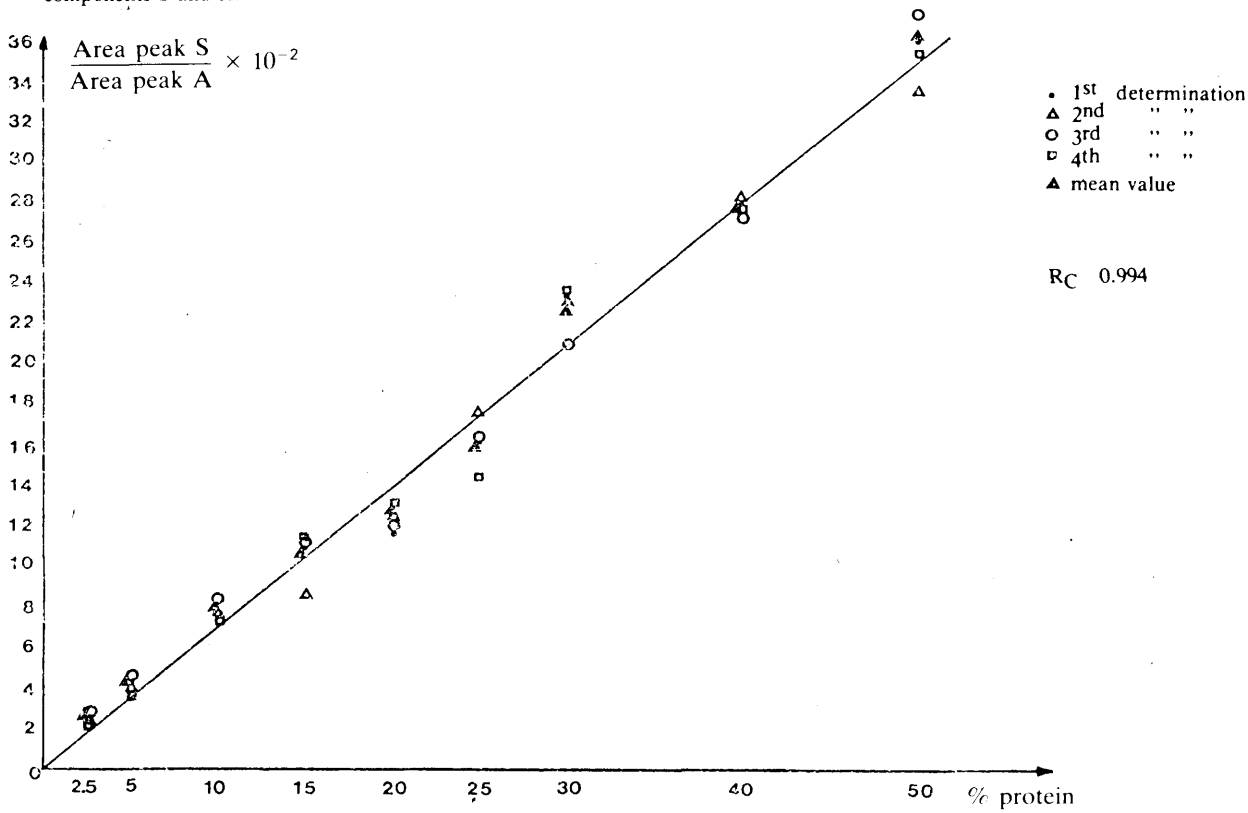
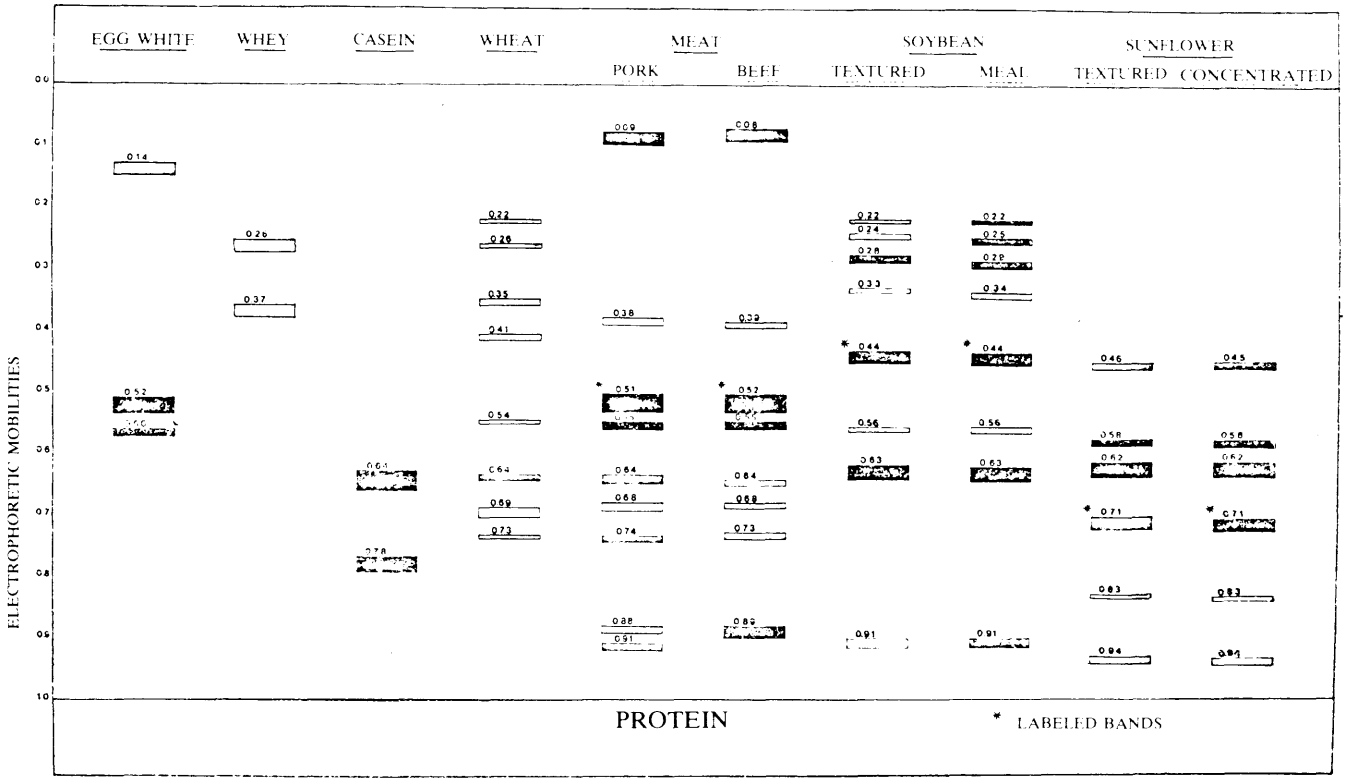


Figura 6.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