Reprinted from the JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY, Vol.56, No.3, Pages:285-287

Methods for Detection and Determination of Vegetable Proteins in Meat Products

W.J. OLSMAN, Central Institute for Nutrition and Food Research, TNO, Postbas 360, Utrechtseweg 48, 3700 AJ Zeist, The Netherlands

ABSTRACT

Soy protein is the vegetable protein that is most frequently used in meat products. Accordingly, detection and determination procedures have mainly been focused on soy proteins. Cereal proteins received far less attention analytically, let alone the less conventional vegetable proteins. Every method published has only a limited applicability, determined by both the type of soy preparation concerned and the heat processing of the sample. The methods may be divided into five categories. 1. Chemical methods are based on analysis of tracer substances accompanying the soy proteins by nature. Their specificity is rather low; other vegetable proteins may contain the same substances. Soy flour, concentrates and texturates respond quantitatively, and sometimes even qualitatively, different. The methods are almost useless for isolated soy proteins. 2. Microscopic methods may allow rapid detection of soy products except isolates. They may be used for quantitation purposes. However, representative results will only be secured at the expense of time and labor. 3. Electrophoresis methods rely on the recognizability of soy protein bands in the pherogram pattern. Field of application and specificity are satisfactory. Efficient media enable complete solubilization of soy protein from meat products, if not severely heat-processed. 4. Immunochemical methods, although very sensitive and specific, are only suitable for detection purposes, provided the sample temperature did not exceed 100 C during processing. This holds, of course, only true if the soy produced used is not excessively heated during preparation. 5. Methods based on amino acid composition or sequence are based on computer matching of the amino acid pattern of the meat product sample with those of varying mixtures of all proteins that could be contained in the sample.

Increasing amounts of soy protein are being up-graded to a wide diversity of protein products for human nutrition. However, soy protein probably will not supersede any of the common protein-rich staple foods in the Western world, such as meat, milk and dairy products, cereals and beans. The soy proteins, therefore, had to acquire a share of the market as protein substitutes or extenders. This evoked the need to regulate their use, and regulations demand tools to ensure their enforcement. Repressive control in the laboratory can only be performed if adequate analytical methods are available.

A substantial part of the soy protein products for human consumption is used in the manufacture of meat products. At present soy protein is the most frequently applied vegetable protein in these products. Accordingly, many methods have been proposed for detection and determination of soy protein in meat products. Cereal and other oil seed proteins, which are also added sometimes, received far less attention from analysts.

The published methods of analysis for soy proteins comprise a diversity of principles. An elaborate survey is given by Olsman and Krol (1). Each has only limited applicability, dependent on both the type of soy preparation concerned and the heat treatment of the meat product. A division into two main groups can be made:

A. Methods based on the presence of substances accompanying the proteins	1. chemical methods 2. microscopic methods
B. Methods based on proteins themselves	1. electrophoretical methods 2. immunochemical methods 3. methods based on amino acid composition or sequence

Substances that have been used as tracers revealing the presence of soy proteins or to estimate their concentration by means of chemical methods are: oligosaccharides such as raffinose, stachyose and verbascose, pentosans, hemicellulose and crude fibre, saponins, the amino acid canavanine, phytine or phytic acid, manganese and magnesium. The specificity of these methods is generally rather poor. Cereals and legumes may also contain some of these compounds. Furthermore, the concentration of tracer compounds in soy products of different origin may vary considerably, whereas some of the substances may also occur in the raw meat materials or in other ingredients.

Microscopic methods are very suitable for screening purposes. They are more specific than the chemical methods because of their showing morphological characteristics. A very rapid method used in The Netherlands takes advantage of the presence of calcium oxalate crystals in the cotyledon cells of the soybean (2,3,4). They can be seen in polarized light as polygonal green colored bodies, as shown in Figure 1. Most microscopic techniques rely on histological staining of the polysaccharide cell wall constitutents of the soy bean. Such a procedure has recently even been advocated for quantitation purposes (5) using

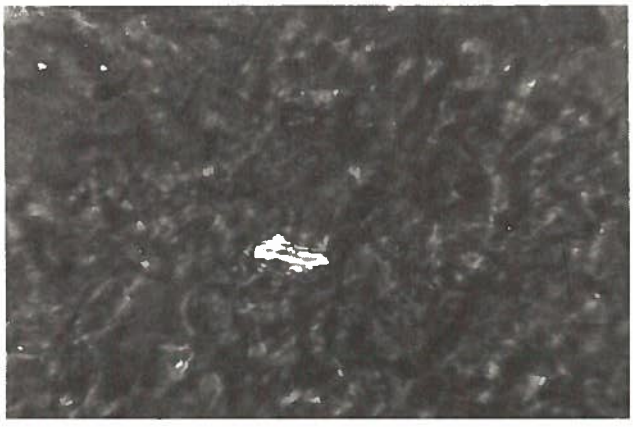

FIG. 1. Ca-oxalate crystals in dried defatted material of a luncheon meat sample containing textured soya protein; magnification 450 x.

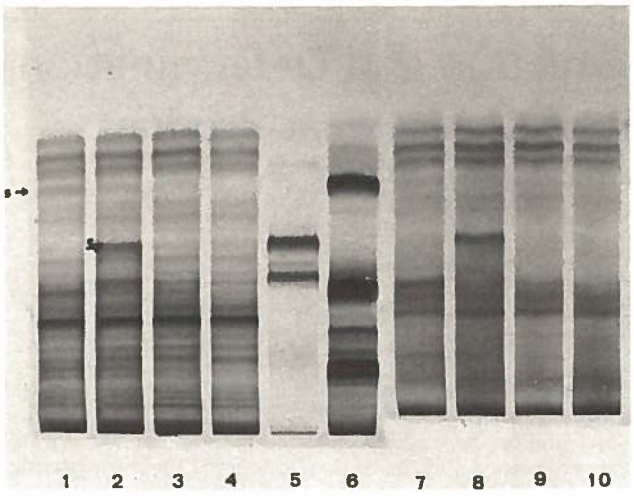

FIG. 2. SDS-Polyacrylamide gel electrophoresis; pherograms of luncheon meat type products: 1-4 pasteurized products (60 min at 85 C) with 2, 1, 0.5 and 0% soy protein isolate; 5 sodium caseinate; 6 soy protein isolate; 7-10 shelf stable products (I_o = 0.57) with 2, 1, 0.5 and 0% soy protein isolate; products 2 and 8 also contain 1% sodium caseinate.

stereo techniques which are necessarily rather time-consuming. Group A methods only apply to soy flour, grits and textured products and – to a slightly lesser extent - to concentrates and textured preparations made from them. Purified proteins, like isolates, solely depend on the B methods for their detection and quantitation, although some substances may partly survive the purification procedure and remain partially associated with the proteins (e.g., phytin).

Electrophoretic methods require the complete solution of proteins for their subsequent separation. When a meat product is heated, the proteins will denature and generally lose their solubility in water or dilute buffer solutions. The protein coagulum of a heat-processed meat product may be considered as a random three dimensional network of intertwining polypeptide chains, which interact by hydrogen, hydrophobic and disulphide bonds. Proteins can adequately be extracted from this mass by using reagents such as detergents, concentrated solutions of urea and SH-reagents. The dissolution is the more successful, as the heat treatment of the product has been milder. Remarkably enough, solubilization of soy proteins from heated meat systems has never been studied systematically as far as we know from the literature. However, elaborate studies have been performed on thoroughly heat-denatured soy bean meal (6), showing that 16% of protein can be extracted with a pH 8.6 buffer solution. The same solution containing 8 M urea and 0.1 M 2-mercaptoethanol dissolves 76% of the proteins. Extraction of the dried, defatted meat product sample with a buffer solution containing sodium dodecyl sulphate (SDS) and 2-mercaptoethanol (ME), followed by electrophoresis in an SDS-containing polyacrylamide gel at slightly alkaline pH, seems to be the best method available today. Figure 2 shows the electrophoretic patterns of pasteurized and shelf-stable luncheon meat containing different levels of soy protein isolate. Soy proteins are detectable down to levels of 1% on whole product, the characteristic soy band marked by the arrow being somewhat less pronounced for the shelf stable samples. Quantitation by densitometry is possible in principle, but the reliability of quantitative data can only be evaluated from collaborative studies which, to our knowledge, have not yet been undertaken. At any rate, electrophoretic quantitation of soy proteins in meat products cooked at temperatures above 100 C seems to be difficult because of reduced extractability.

Immunochemical methods are characterized by high specificity and sensitivity. Nevertheless, their field of application is limited for various reasons.

In order to maintain their antigenic properties, soy proteins should be extracted from meat products only under relatively mild conditions, which may not allow their complete dissolution. It is true that in current antiserum production procedures, part of the immunogenic soy protein is subjected to heat prior to administering it to rabbits, in order to elicit antibodies against the heat denatured proteins present in heat-processed meat products. However, sample extraction with urea or SDS would bring on additional irreversible changes in the protein conformation of the soy antigens so that they would lose the ability to form complexes with their antibodies. These circumstances hamper the achievement of favorable conditions for quantitative work.

Differences in processing soy proteins at various factories, and perhaps genetic varieties of the soy bean as well, affect the determination of soy proteins by any immunochemical method. According to Hammond et al. (7) many textured soy products do not respond to antisoy isolate serum. Hauser et al. (8) found the antiserum to promine D to react with its homologous protein antigen only, and not with four other commercially available soy protein isolates. The observation is supported by experiences with soy isolates, gained at the former Animal and Plant Health Service of the U.S.D.A. It was found that antisera, produced with any batch or lot of isolated soy immunogen, would not necessarily react against other batches or lots.

The third factor limiting the widespread use of immunochemical methods is the fact that antisera of consistent quality, with specified high titres, are difficult to obtain. Antisoy serum is commercially available; however, its titre is relatively low, variable and not specified. There is an urgent need for a better and more defined antiserum. Some investigators, for that reason, prefer to prepare their own antisera. Close cooperation and exchange of ideas between food analysts and (potential) suppliers of antisera – provided they are sincerely interested in the relatively small market – would be necessary to achieve a substantial improvement in the present situation. For the time being, immunochemical methods are only of limited use for the raw and mildly heated meat products for the presence of soy proteins. Quantitation by these methods is impossible.

The group of methods based on amino acid composition or sequence comprises two novel approaches. Digestion of the protein mixture from meat product samples with the proteolytic enzyme trypsine (9,10,11,12) gives a peptide mixture which can be subjected to ion exchange chromatography. The complex elution pattern shows a small but distinct peak, mainly originating from the 11 S-fraction of the soy protein complex. It appeared that about 60% of the peptides constituting the peak could be attributed to one single pentapeptide. It may be questioned if this elegant method is not too sophisticated for use as a routine method for the determination of soy protein content in meat products. Furthermore, no information is given as to whether the result could be affected by the presence of other nonmeat proteins in a meat product sample. From tentative experiments in our institute, we concluded that erroneous results may be obtained for meat products containing casein.

The fact that every protein has its own characteristic amino acid pattern offers a key to developing the identity of protein components in mixtures and the latter's quantitative composition. For long this was merely an interesting idea. In 1975, however, the computer was used to match the amino acid pattern of a food product sample with those of proteins that come into consideration as possible constituents of that particular food product (13). Whereas Lindqvist (13) fixed his attention to dairy products, we in our institute

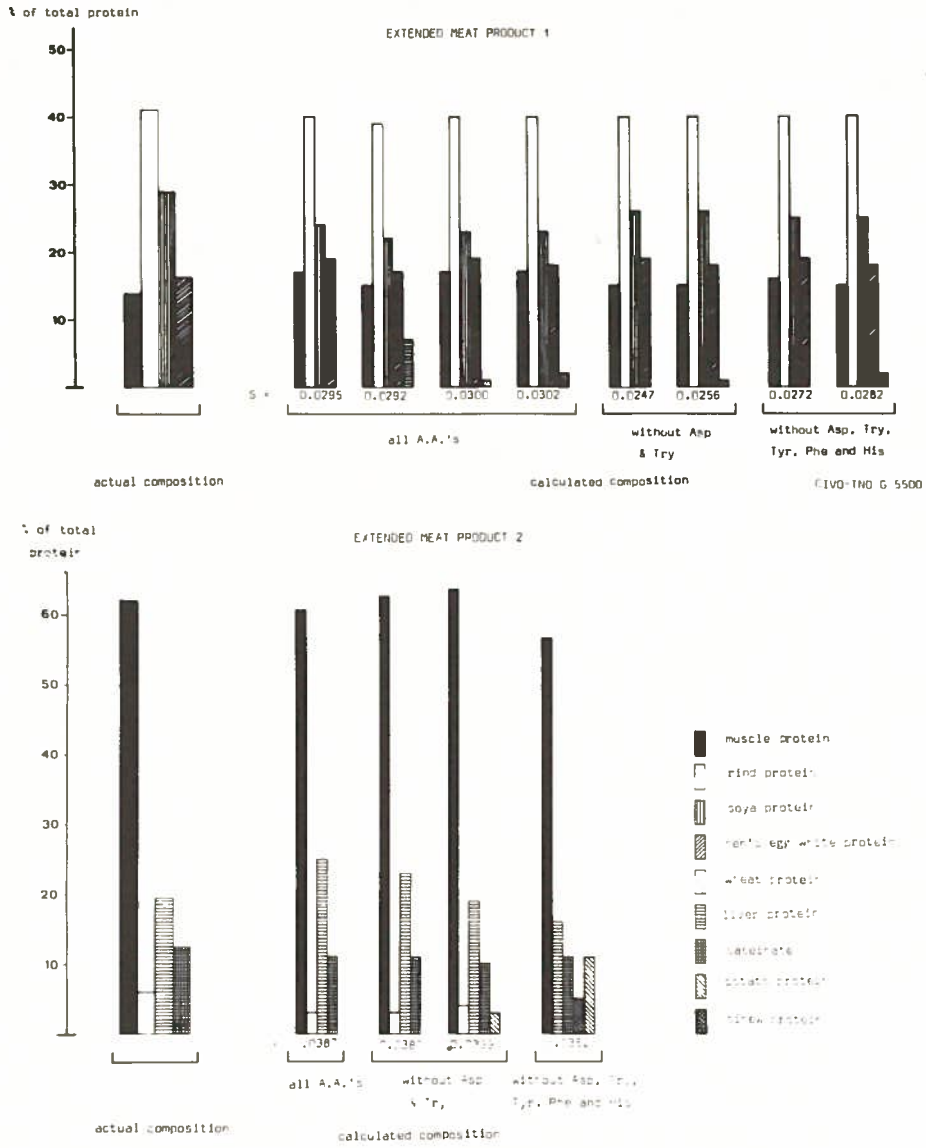

FIG. 3. Actual protein composition and the most probable compositions as calculated by computer evaluation of the amino acid pattern for two extended meat products; s = standard deviation from regression of amino acid data.

are evaluating the feasibility of a similar multiple regression procedure to identify – qualitatively as well as quantitatively – the different protein ingredients in meat products. Figure 3 shows results of two pasteurized comminuted meat products, each containing four protein ingredients. For commercial meat products, which may contain protein hydrolyzates, a preliminary removal of low molecular N-containing compounds by extraction with a trichloroacetic acid solution is necessary. Although this approach exceeds the scope of a specific soy protein method, it should not be omitted from this survey because of the advantage of being capable, in principle, to produce a complete picture of the protein composition in one procedure. None of the great variety of methods of analysis for soy proteins in meat products has yet been generally accepted as the best or the most promising. Such judgments can only be made on the basis of results of comparative tests in several laboratories. To our knowledge such studies have hardly been undertaken up to now. However, they are prerequisites for the development of standard methods of analysis.

REFERENCES

1. Olsman, W.J., and B. Krol, "Methods for the Detection and Determination of Vegetable Proteins in Meat Products," Appendix VII of the Report of the Study Group on Vegetable proteins in Foodstuffs for Human Consumption, in Particular in Meat Products, C.E.C., Brussels, April 1978, pp. 112-140.
2. Czaja, A. Th., Forschung 127:17 (1965).
3. Moosdijk, A.J.E. van de, and M.E.C. Werten, Ware(n)-Chemicus 6, p. 204, (1976).
4. Schut, K., Ware(n)-Chemicus 6, p. 214 (1976).
5. Flint, O., and M.C. Meech, The Analyst 103:252 (1978).
6. Shibasaki, K., K. Okubo, and T. Ono, J. Food Sci. Technol. (Tokyo) 16:22 (1969).
7. Hammond, J.C., I.C. Cohen, J. Everard, and B. Flaherty, J. Assoc. Public. Anal. 14:119 (1976).
8. Hauser, E., J. Bicanova, and W. Künzler, Mitt. Geb. Lebensmittelunters. Hyg. 65:82 (1974).
9. Bailey, F.J., J. Sci. Food Agric. 27:827 (1976).
10. Bailey, F.J., and C.H.S. Hitchcock, Ann. Nutr. Alim. 31:259 (1977).
11. Bailey, F.J., J.W. Llewellyn, C.H.S. Hitchcock, and A.C. Dean, Chem. Ind. 177 (1978).
12. Llewellyn, J.W., A.C. Dean, R. Sawyer, F.J. Bailey, and C.H.S. Hitchcock, J. Food Technol. 13:249 (1978).
13. Lindqvist, B., J. Ostgren, and I. Lindberg, Z. Lebensm. Unters. Forsch. 159:15 (1975).