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Afdeling Eiwitchemie 1983-10-12

RAPPORT 83.75 Pr.nr. 505.7010

Projekt: Ontwikkeling methoden voor het aantonen en bepalen van vleesvreemde eiwitten

Onderwerp: Quantitative methods for differentiation of vegetable and animal proteins in foods II.

Voorgaand verslag: 82.43.

Doel:

Op verzoek van de nederlandse delegatie van het Codex Committee on vegetable proteins (CXVP) is een tweede "working paper" over de differentiatie van plantaardige en dierlijke eiwitten in voedingsmiddelen samengesteld.

Samenvatting:

Deze "working paper" is een vervolg op de eerste van november 1981 over hetzelfde onderwerp (RIKILT-verslag 82.43). Ze beschrijft welke vooruitgang het kwantitatieve onderzoek van eiwitten van plantaardige en dierlijke oorsprong gedurende de afgelopen twee jaar heeft gemaakt.

Conclusie:

In de afgelopen twee jaar is niet veel vooruitgang geboekt met dit onderwerp.

Men is bezig geweest om enerzijds oude methodieken te optimaliseren, zoals toepassing van de ELISA-techniek en kwantificering via secundaire parameters (fytaat-fosfor en suikers voor plantaardige componenten), anderzijds nieuwe doch kostbare methoden te ontwikkelen waarvan de algemene bruikbaarheid in de praktijk nog moet worden bewezen.

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Projektleider : H.L. Elenbaas

WORKING PAPER FOR CODEX COMMITTEE ON VEGETABLE PROTEINS (CXVP) ON:

QUANTITATIVE METHODS FOR DIFFERENTIATION OF VEGETABLE AND ANIMAL PROTEINS IN FOODS. II

prepared by W.J. Olsman*, H.L. Elenbaas** and W. de Groot***

INTRODUCTION

The food technologist is increasingly active in optimizing the functional properties of more or less traditional food products within the constraints set by the market situation. It is in the interest of the different branches of agricultural production and the associated industry, as well as the consumer that sound analytical methods become available to establish the "composition" of the final product. That is, to enable public analysts to determine to which extent it is derived from the basic produce of agriculture, e.g. meat, milk, egg, wheat, soya, potatoe etc.
The protein fraction of a product bears a clue for such a differentiation.
This paper is a follow-up to the first working paper of November 1981 on this subject. It describes the progress, made in the last two years.

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X

DIFFERENTIATION TECHNIQUES FOR MEAT PRODUCTS

The ISO-working group for the detection and determination of non-meatprotein in meat products (ISO/TC 34/SC 6/WG 7) met in September 1982. A report of that meeting is attached to this paper.

1. Soya proteins

Quite recently, some initiatives have been taken in Europe to carry out ring tests of some promising methods. The number of positive responses from the invited participants substantiates that, despite budgetary restrictions, there is an increasing awareness of the value of such test-programmes. A final evaluation of methods can only be made on the basis of the results of collaborative studies. In a key article published in 1981, Hitchcock et al (1) introduced the Enzyme-Linked Immunosorbent Assay (Elisa) for the determination of soya protein in food, specifically meat products. Results from the first cooperative trials in some laboratories warranted further testing in a collaborative study, organized by the U.K. Ministry of Agriculture, Fisheries and Food. The same was done on a European level by Euvepro. In addition, an electrophoretic technique was ringtested with the same pasteurised meat product samples. The latter method, published in 1982 by Armstrong et al (2), contained a new element: the use of an internal-standard protein. The first impression from the recently collected results is, that the better quantitative results are obtained with the Elisa-approach. The final results will be published in due course.

Griffiths et al (3) reviewed three modern techniques for determining soya proteins in meat products: microscopical stereology, the peptide and the Elisa approach. The latter appeared to be the cheapest method, both in man hours per determination and equipment costs. The peptide approach produced the most accurate results, but was highest in investment costs. The authors consider it necessary for the time being to rely on more than one approach. A quick microscopical examination for determining which kind of soya product is present, seems a logical procedure to start the examination of the sample.

Simple indirect chemical methods, though not very specific, may be useful for screening purposes. The phytic acid approach belongs to this category. Using this technique, a Dutch working group recently obtained encouraging results after more rigidly standardising the analytical procedure, as proposed by the Unilever Research Laboratory at Vlaardingen (NL). (4)

Morrissey et al (5) published a method based on the relatively high content of galactose and arabinose in the polysaccharide fraction of soya products. Even in isolates, the level is a hundred times higher than in meat. The monosaccharides are released by hydrolysis and determined enzymatically with galactose dehydrogenase and NAD^+.

A weak point of all indirect methods is the variability of the relation between the contents of the non-protein tracer compound and the soya protein.

2. Gluten and other vegetable proteins

The second most important plant proteins for application in meat products is wheat gluten. It may well be possible that the progress made nowadays in the study of wheat proteins, will yield methods for differentiation of gluten in meat products in the near future. No data are available on methodology for other vegetable proteins.

3. Meat proteins

In the light of considerations in various countries to set minimum levels to the muscle meat content of meat products, the recent progress in the methodology of determining protein-bound 3-methylhistidine as an index for this purpose should be memorised (6). If this approach would eventually develop into a routine method, the need for protein differentiation may become less of a problem.

4. New possiblities?

A multivariate analysis of the amino acid composition of an extended meat product sample has been mentioned as a promising method to determine its protein composition (7).

LITERATURE

In addition to the publications, mentioned in the text above, the literature list also contains other articles of the last two years, that deserve mentioning.

1. Hitchcock, C.H.S., Bailey, F.J., Crimes, A.A., Dean, D.A.G. and Davis P.J., Determination of Soya Proteins in food using an Enzyme-linked Immunosorbent Assay procedure.
   J. Sci. Food Agric. 32 (1981) 157-65.

2. Armstrong, D.J., Richert, S.H. and Riemann, S.M., The determination of isolated soyabean protein in raw and pasteurized meat products.
   J. Food Technol. 17 (1982) 327-37

3. Griffiths, N.M., Billington, M.J. and Griffiths, W., A review of three modern techniques available for the determination of soya protein in meat products.
   J. Assoc. Publ. Analysts 19 (1981), 113-9.

4. Unpublished method developed at Unilever Research Vlaardingen, The Netherlands.

5. Morrissey, P.A., Olbrantz, K and Greaser, M.L.
   A simple sensitive enzymatic method for quantitation of soya protein in soya meat blends.
   Meat Science 7, (1982) 109-16.

6. Jones, D., Thorley, D and Hitchcock, C.H.S.
   The fluorimetric determination of 3-methylhistidine in meat and meat products.
   J. Sci. Food Agric. 33 (1982) 677-85.

7. Olsman, W.J., Slump, P. and Thissen J.
   Computer aided determination of the protein composition of extended meat products.
   Proceedings 27th Meeting of European Meat Research Workers, band II, p. 54-58, Vienna 1981.

8. Klostermeyer, H. Quantitative determination of milk and non-milk proteins in products covered by the FAO/WHO code of principles.
IDF, E-Doc. 185/83.

9. Ring, Ch., Weigert, P und Hellmannsberger, L.
Zur Differenzierung von Eiweiss pflanzlichen und tierischen Ursprungs mittels Disk-Polyacrylamid-Gel-Elektrophorese.
Fleischwirtschaft, 62 (1982) 648-50.

10. Ring, Ch und Sacker, F.
Zum quantitativen Nachweis von Sojaprotein in hoch erhitzten Fleischerzeugnissen.
Proc. 28th Eur. Meet. of Meat Res. Worker, Madrid 1982, pp. 436-438.

11. Hellmansberger, L.
Routine detection of foreign proteins in heated meat products by polyacrylamide disc electrophoresis.
Thesis, Ludwig-Maximilians-Universität, München, 1981.

12. Molander, E.
Determination of soya protein in meat products by standard curves obtained from gel electrophoresis.
Z. Levensm. Unters. Forsch., 174 (1982) 278-281.

13. Janssen, F.W. and De Baay, J.A.
A contribution to the electrophoretic investigation of meat products on the presence of non-meat proteins or on species falsification using SDS-MZE electrophoresis.
De Ware(n) Chemicus, 12 (1982) 176-84 (in Dutch).

14. Eldridge, A.C., Determination of Soya protein in processed foods.
J. Am. Oil. Chem. Soc. 1981, 483-5.

15. Menzel, E.J. and Glatz, F.
Die radioimmunologische Bestimmung von nativem und hitzedenaturiertem Sojaprotein.
Z. Lebensm. Unters. Forsch., 172 (1981) 12-19.

16. Hofmann, K.

Zur Problematik der Bestimmung von Sojaeiweiss in Fleischerzeugnisse. Mittelsblatt der Bundesanstalt für Fleischforschung, 87 (011282) pp. 5299-5301.

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ISO/TC 34/SC 6/WG 7
Title: Meat and meat products
- Determination of non meat proteins

Secretariat: NNI (Netherlands)

The second meeting of the ISO Working Group for the detection and determination of non-meat proteins in meat products /ISO TC 34/ SC 6 WG 7/ was held on 24th September in Balatonfüred /Hungary/. The meeting approved the second report / 34/SC 6/ N 223/ and the third report / 34/SC 6/WG 7/ N 01 /

A. Recent collaborative studies.

1. The Netherlands. The following studies are in progress :

1./ Casein : Laurell /rocket/ electrophoresis / 8 laboratories, 3 tests of 15 samples each; range of casein content 0.5 - 2.5 % /. First results are encouraging.

1.2 Soya Protein: phytate via its phosphorus content / 11 laboratories 5 samples; range of soya protein content / 2.5 - 4.5 %/. Unsatisfactory results were obtained. Another collaborative test is planned, based on an improved procedure.

1.3 Soya Protein : detection by SDS-PAGE /10 laboratories/. To be investigated as a quantitative densitometric / Purina/ procedure.

1.4 Soya Protein: detection of soya meals by microscopy.

2.U.K. Since 1980, considerable progress has been made : three methods have been published: a./ Quantitative SDS- PAGE/ Armstrong et al., J. Fd. Technol. 1982. 17, 327-337/.

/b/ ELISA /Hitchcock et. al. J. Sci. Fd. Agric. 1981. 32. 157-165/.

/c/ Mettis as an index for muscle protein / Jones et. al., J. Sci. Fd. Agric 1982. 33. 677-685/.

There is no confidence in indirect and electrophoretic methods .

The quantitative histological method / Flint & Meech, Analyst, 1978, 103,252 /has been ring-tested by public analysts: the results are not yet available but are not satisfactory. One public analysts' laboratory has compared three methods /histology, peptide and ELISA/; the last two were promising and ELISA was recommended for its cost/convenience / Griffiths et. al. J. Assoc. Publ. Analysts. 1981. 19 113-119/. A Government Ring test of ELISA is planned using samples based on U.K. products; the results are expected in mid 1983. At the same time Euvepro are planning to test the ELISA and SDS-PAGE to be organised by Dr,W.J. Olsman / convenor of WG 7/. The ELISA protocol has been drawn up in ISO format and will be made generally available.

3 France. Histology is used exclusively. France has been already approached by Euvepro and is willing to test ELISA but not SDS-PAGE.

4 U.S.A major research programme is planned by the USDA, but no results are yet available. Details of some relevant AOAC methods are attached.

5. Czechoslovakia. Soya is not used; wheat flour and caseinate is used but no techniques are available.

6. Hungary. Soya and caseinate are used but seldom detected. /using an electrophoretic method. /

B. New Methodology.

1. France . A histological method is available for the quantitative detection of insoluble vegetable protein. Attempts to quantify this technique using an image analyser with skilled operators have been successful but prohibilitively expensive.

2. Czechoslovakia. A spectrophotometric determination of creatinine/ creatine was being developed as an index for meat.

3. U.K. Creatine suffers from drip loss and decomposition on heating in some products, and is a measure of meat extracts, while methyl-histidine is a measure of lean meat protein, however, methyl histidine is not appropriate to examine small quantities of non-meat proteins due to its inherent variability.

4. France Products with low meat levels could be analysed for meat via methylhistidine and hydroxyproline allowing the non-meat protein to be calculated by difference with sufficient accuracy.

5.U.K. Other methods which are not promising include HPLC for peptides,comparison of amino acid profiles, enzymic estimation of galactose/arabinose, fluorescent markers.
Information was tabled that Euvepro planned to organise a demonstrati meeting of the ELISA technique at a convenient venue /tentatively scheduled for December 8 th 1982 at Schiphol/. Anyone willing to cons contributing to the Euvepro ring test is welcome to contact Euvepro. Rue de l'Orme, Bruxelles, Belgium /.

C. Recommendations

1. WG 7 should await the results of the forthcoming Euvepro ring test and plan a further ISO test depending on the outcome.

2. WG 7 should follow the progress of research in the U.K. and Netherlands designed to establish methyl histidine as a meat index.