Afd. Vlees en Vleesprodukten

Verslag 82.43 1982-05-07

Pr.nr. 505.7010

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

Verzendlijst: direkteur, direktie VKA, sektorhoofd (3x), afd. Vlees en Vleesprodukten(4x), afd. Normalisatie (Humme), Projektbeheer, projektleider.

Afdeling Vlees en Vleesprodukten

Datum: 1982-05-07

Pr.nr. 505.7010

VERSLAG 82.43

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

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

Doel:

Op verzoek van de nederlandse delegatie van het Codex Committee on vegetable proteins (CXVP) hebben twee leden van de werkgroep MOVE in samenwerking met het CXVP (Van Gils) een working paper opgesteld over geschikte kwantitatieve methoden voor de differentiatie van plantaardige en dierlijke eiwitten in voedingsmiddelen.

Samenvatting:

Na het stellen van een aantal uitgangspunten voor een analytische methode wordt een overzicht gegeven van de analysemethoden voor de differentiatie van eiwitten in voedingsmiddelen van dierlijke (vlees en zuivel) en van plantaardige oorsprong (bakkerijproduken). Het accent van deze studie ligt op de bepalingsmethoden van toegevoegde plantaardige eiwitten.

Conclusie:

Een enkele methode voor de bepaling van plantaardige eiwitten in allerlei soorten voedingsmiddelen zal voor altijd een utopie blijven. In principe is de SDS-polyacrylgelelektroforese nog de meest universele methode. De methode geeft nog geen voldoende reproduceerbare resultaten om haar voor te stellen als een standaard analysemethode. Heden ten dage en in de nabije toekomst zijn soja-eiwitten en in beperkte mate ook tarwe-eiwitten (gluten) de enige plantaardige eiwitten die beschikbaar zijn voor de voedingsmiddelenindustrie.

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

Quantitative methods for differentiation of vegetable and animal proteins in foods

Prepared by : W.J. Olsman, H.L.Elenbaas and H.W. van Gils

I INTRODUCTION

Modern food manufacturing makes it possible to market protein substitutes or enriched food products based on traditional commodities.
Particularly in the case of such commodities as meat, dairy and bakery products, it should be possible to the public analyst to determine whether proteins have been added, which proteins and how much.
Although theoretically any mixture of proteins may be marketed, for practical reasons this report has been restricted to above mentioned commodity groups and to the quantitative determination of vegetable proteins, such as soya proteins and of wheat gluten additions to such food products. Other protein products such as derived from rapeseed, sunflower, field beans etc. may have a future, but today they are practically not available for food-manufacturing and are therefore not to be considered in this report.

II POINTS OF DEPARTURE

Requirements for an analytical method for purposes of enforcement of regulations:

- Vegetable proteins should qualitatively be determinable in order to distinguish the "pure" product from products containing extraneous proteins.
- Extraneous proteins should also be quantitatively determinable, because any legally permitted addition will be restricted. Moreover a quantitative determination will be necessary to assess the correctness of the labelling.
- The analytical method must have an acceptable accuracy. Although no tolerance can be given yet, a 95 % confidence limit of more than 40 % of the average result will certainly not be acceptable.

Although scientifically it may be possible to differentiate quantitatively between most vegetable and animal proteins this report is restricted to those methods which are considered useful tools in the hand of the public analyst.
This implies, that those techniques that are cumbersome or require highly skilled personnel are only mentioned when they stand a chance for automation or simplification without extreme capital investments.

- The determination of the extraneous protein content should preferably be possible without pre-knowledge of the type of material and methods of manufacture involved. The public analyst usually samples ready products and does not visit the factory, certainly not in case of imported products.
- The execution of the analytical method should be possible with apparatus and personnel as usually is available at public inspection laboratories. No extreme extra costs should be involved in the regular application of the method.

III REVIEW OF METHODS

There is no single analytical method with general applicability to protein differentiation in food products. The choice of the procedure will be determined predominantly by the specific problem to be solved, such as the analysis of protein-enriched flours, mixed products on dairy basis, or heat processed meat products for non-meat proteins.

A method with a wide field of application should necessarily be based on properties of the proteins themselves. Two categories of methods meet this criterion : the immunologic and the electrophoretic methods. The first seems to be useful only for detection purposes, (due to the fact that the antigenic properties vary widely, depending on differences in processing conditions and breed). The remaining category of electrophoretic methods are applicable only as far as the proteins can be brought into solution. This implies, especially in the case of heated products, the use of extractants capable of abolishing intra- and intermolecular protein interactions (e.g. solutions containing urea or sodium dodecyl sulphate, SDS.) The technique of electrophoresis in polyacrylamide gels containing SDS (or SDS-PAGE), finding ever increasing acceptance, seems to be the method of choice, in our opinion.

Stating this should not give rise to the idea that this method is a panacea for the whole area of problems of protein differentiation. This was shown to be true even for the limited problem of determing the soya protein content in meat products, by the results of collaborative work of some 10 Dutch laboratories. SDS-PAGE does not yet meet the requirements of reproducibility which are obligatory for standard methods of analysis.

Extractants containing SDS, are not always capable of dissolving the proteins completely before applying electrophoresis. Particulary for wheat proteins (gluten) and severely heated soya proteins quantitative determination by SDS-PAGE due is not possible to their insolubility.

IV DIFFERENTIATION TECHNIQUES FOR MEAT PRODUCTS

The vast majority of methods hitherto published deal with the problem of detection and determination of non-meat proteins in meat products. Some review articles have appeared recently, and an ISO expert working group is envisaging the possibilities of proposing draft standard methods of analysis (see paper enclosed). The final clause of the 1980 progress report of this ISO-group was moderately optimistic. This optimism has meanwhile been refuted by disappointing results of collaborative work in the Netherlands.

1. Soya proteins

These proteins can be extracted from raw and moderately heated (up to 100 °C) meat products, and subsequently be subjected to SDS-PAGE. Different groups used this technique with some success.

2. Gluten. No suitable methods available.

3. Other vegetable proteins : no information.

V DIFFERENTIATION TECHNIQUES FOR DAIRY PRODUCTS

Since in many countries admixture of vegetable proteins to dairy products is legally prohibited, very few quantitative differentiation methods are worked out yet.

Soya proteins:

These proteins can be extracted from moderately heated dairy products and determined quantitatively by SDS gel electrophoresis. Also starch gel electrophoresis is described as a useful technique to determine milk proteins in animal feeding stuffs containing soya proteins.
Adequate determination techniques for sterilised or fermented dairy products have not been described in literature.

Gluten:

No quantitative determination methods adequately meeting the requirements as described in this paper have been described in literature.

Other vegetable proteins:

No data available.

VI DIFFERENTIATION TECHNIQUES FOR BAKERY PRODUCTS

Addition of gluten to bakery products is not considered to fall into the terms of this working paper and therefore only techniques to determine soyaproteins in bakery products have been considered.

VII SUMMARY

In principle, SDS-PAGE is the method having the widest applicability. However, methods of analysis employing this technique, up to now do not yield sufficiently reproducible results to justify the drafting of a standard method of analysis.
More research work will be necessary for this. One single procedure for the analysis of vegetable proteins in all kinds of food products will be Utopia forever, irrespectable of the progress being made in the future.

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November, 1981