## Detection of Milk- and Soya-proteins in Meat-products

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Milk- and soya-proteins, worked into meat-products, were separated from muscle-proteins by the use of polyacrylamide gel disc electrophoresis. Protein fractions were stained by Amido-Black dye and identified by their relative mobility.
A developing method, suitable for rapid qualitative determination was elaborated by the application of which the composition of samples could be evaluated in considerably shorter time, 3–4 hours after the finishing of electrophoresis. According to this method in the course of oxidation by sodium periodate followed by reduction with sodium bisulphite, iodine precipitation was greater on bands of milk- and soya-protein fractions than on the background.
Quantitative determination of milk- and soya-proteins was realised by densitometric evaluation, using Fast-Green dye and special standard samples. Both, the quantitative and qualitative determinations are suitable for routine work.

## Nachweis von Milch- und Sojaeiweiss in Fleischwaren

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Das in die Fleischwaren eingearbeitete Milch- und Sojaeiweiss wurde mittels Polyacrylamidgel-Disk-Elektrophorese vom Muskeleiweiss getrennt. Die Eiweiss-Fraktionen wurden mit Amido-Black Farbstoff gefärbt und auf Grund ihrer relativen Mobilität identifiziert.
Eine für die schnelle Qualitätsbestimmung geeignete Entwicklungsmethode wurde ausgearbeitet wodurch die Zusammensetzung der Probe während einer wesentlich kürzerer Zeit als üblich, schon innerhalb von 3–4 Stunden nach der Beendigung der Elektrophorese ausgewertet werden konnte. Das Wesentliche an der Methode ist, dass bei der Reduktion des Natriumbisulfits nach der Oxydation des Natrium-perjodats die Jodausscheidung an der Stelle der Milch- und Sojaeiweiss-Fraktionen grösser ist, als im Hintergrund.
Die quantitative Bestimmung der Milch- und Sojaproteine wurde durch die Anwendung des Fast-Green Farbstoffes densitometrisch durchgeführt. Zur Bestimmung sind spezielle Standardproben angewendet worden. Sowohl die quantitative als auch die qualitative Bestimmung kann als Routinemethode durchgeführt werden.

# 5.26

## La détection de la protéine de lait et de soja dans les produits de la viande

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Les protéines de lait et de soja, employantes dans les produits de la viande, elles ont été séparées de la protéine du muscle par électrophorèse de gelée poliacrilamide. Les fractions de protéine ont été colorées par le colorant Amido - Black et identifiées sur la base d'une mobilité ressemblée au contrôle.
Nous avons développé une méthode pour déterminer rapidement la qualité avec laquelle il peut être appréciée la composition d'échantillon, c'est-à-dire dans un temps plus court que d'habitude, en 3-4 heures de la fin de l'électrophorèse. C'est le principe de cette méthode qu' au cours de la réduction de natriumbisulfide qui suit l'oxydation de natriumperiodathe aux lieux des fractions de protéine de lait et de soja la séparation d'iode est plus grande que celle en arrière-plan.
La détermination quantitative des protéines de lait et de soja a été réalisée par une exploitation densithomètre, employant le colorant Fast-Green.
Au cours des déterminations nous avons utilisé des échantillons spécials, standardisés. Ainsi que la détermination quantitative, même que celle qualitative peuvent être réaliser comme une méthode de routine.

## Выявление молочных и соевых белков в мясных продуктах

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Молочные и соевые белки, вработанные в мясные продукты, были отделены от мышечных белков с помощью дискового электрофореза в полиакриламидном геле. Белковые фракции были окрашены краской "Амидо-Блэк" и идентифицированы по их подвижности в сравнении с контролным образцом.

Авторы разработали быстрый метод проявления для качественного определения, который позволил провести оценку составов образцов через 3-4 часа после окончания электрофореза, т. е. за время значительно кратче обычного. Сущность метода заключается в том, что в результате окисления периодатом натрия и последующим восстановлением бисульфитом натрия на месте локализации соевых и молочных белков выделение йода более значительное чем на фоне. Количественное определение молочных и соевых белков - при использовании краски "Фаст-Грин" - было осуществлено с помощью денситометра. К определению были использованы специальные стандартные образцы. Как количественное так и качественное определение может выполняться рутинным методом.

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# Detection of Milk and Soya Proteins in Meat-products

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The use of milk and soya proteins as ingredients became wide-spread recently in the meat industry. The good emulsyfying, water and fat holding capacity qualifies them for solving various technological problems. The possibility of their application is extended, however, by the fact of being originated from natural foodstuffs and when applying convenient processing methods they are not to contain substances injurious to health. However, to differentiate them from meat proteins - within them from muscle proteins - is of considerable importance regarding the biological value, the possible differences in storability and some further elements. That's why the elaboration of methods suitable for their quantitative and qualitative detection became necessary.

Numerous methods are described in literature for qualitative detection of milk and soya proteins. There are some methods known also for the quantitative determination, mostly based on immunodiffusion and electrophoresis /1-5/. These methods, however, are not suitable for routine application because of needing special equipment or being extremely laboursome.

After having considered different methods, polyacrylamide-gel electrophoresis was found to be most convenient for the detection of milk and soya proteins in meat products. Electrophoretic patterns of different cuts of beef and pork were compared; the effect of heat treatment and of storage in deep freezer /-18 °C/ on the patterns was studied, too /6/. Casein and milk powder were examined; from among the appearing two highly intensive fractions principally the -casein fraction was found to be characteristic of milk proteins /Fig. 1./. The presence of -casein fraction may be convincing only in great amounts or together with the -casein fraction because its mobility coincides with a beef fraction of weak intensity. Investigation of soya proteins was started by the electrophoresis of Promine-D /Fig. 2./ and the electrophoretic patterns obtained were compared to characteristic patterns of other soya products as textures, isolates, soya flour, too /Fig. 3./. For dissolving the soya products 8 M urea solution proved to be convenient. A characteristic fraction or fraction-group could be observed in the electroferograms which appeared in all soya products and coincided neither with casein nor with meat fractions. Considering the results of these investigations, the quantitative determination of milk and soya proteins in meat-products were performed as follows.

Fresh or deep frozen /-18 °C/ stored and defrosted samples were thoroughly minced, heat treated on 74 °C and homogenized in water or in urea solution. The heat treatment diminishes by denaturation of a part of meat fractions, the number of protein fractions appearing in the gel and facilitates thus the detection of non-protein fractions /Fig. 1./.

In the case of the detection of milk protein fractions, samples were homogenized in distilled water, in the case of soya proteins 8 M urea solution was used. /Although milk

protein may be detected, too, in an urea solution, the use of it seems practical only when soya proteins may be present in the sample because the number of milk protein fractions increases in this medium/. In aqueous solution, however, the release of milk proteins is not quantitatively complete, the amount obtained is safely sufficient for the determination.

Polyacrylamide-gel electrophoresis was performed in Ornstein - Davis system /7/, applying slight modifications /6/. When using urea solutions, both gels and electrod buffers contained urea, too. Fractions were identified by their relative mobility after having made visible by Amidoblack dye.

The disadvantage of the reported method is that although the preparation of the samples and the electrophoresis itself may be performed in a day, evaluation is possible only after 48-72 hours because the necessary destaining of the gels. Sometimes, however, the quick detection of milk or soya proteins might be necessary; so our aim was to work out a method by which the separated protein fractions could be made visible in considerably shorter time.

As the acquisition of Remasol Brilliant Blue dye - which is suitable for this method - was hindered, this could not be used for routine determinations. In our newly elaborated method protein fractions were fixed by a mixture of methanol-acetic acid-water. After this, gel rods were treated with 1 % sodium periodate solution and immersed in a 2 % potassium metabisulfite solution. In the gel rods which became thus yellowish-brown coloured, the colour of iodine weakens after 5-10 minutes, only the protein fractions remain well coloured. Identified on the basis of their relative mobility, they give the characteristic patterns of soya protein or casein. The developed colour is not stable, it can be well detected within 10 minutes, but disappears later. This time, however, is sufficient to measure the place of fractions allowing thus their identification.

The deeper colouring of protein fractions as compared to the background, can be explained by the higher iodine quantity present. This may be the result either of the reducing effect of the proteins or of the higher periodate absorption of them or of both pathways.

This staining method - although not suitable for quantitative determination - is rather advantegous for making rapid decision whether the analysed sample does or does not contain milk or soya proteins. The necessary time for the implementation is 3-3,5 hours from the beginning of the fixing.

The quantitative determination of milk and soya proteins was carried out, basically, similarly to the qualitative determination but the quantification of the method necessitated some essential alterings.

Contrary to the qualitative type, the determination of both milk and soya proteins was performed in urea solution, with gels and buffer also containing urea.

Gel concentration was rised from the former 5 % and 7 % to 9,4 %, thus increasing the accuracy of the determination because of the better separation.

By quantitative determination aqueous caseinate and 8 M urea Promine-D solutions could not

be applied. Considering the codenaturation taking part between meat and non-meat proteins in the course of heat treatment, a process the balance of which is not terminated instantly after the ending of heat treatment, we found necessary to use special standards for the quantitative determinations. The standard series was made up from meat samples containing different amounts of sodium caseinate or Promine-D, the mixture heat-treated on 74 °C for 150 minutes, cooled and stored for at least 3 days in deep freezer. Naturally, when we wish to investigate products processed with considerably different heat treatment, we may modify the heat treatment of the standard samples according to this.

By quantitative determination Fast-green dye was applied because of its giving proportional colour intensity - in defined concentration region - to the concentration of the studied proteins. Quantitative determination was carried out densitometrically using a Chromoscan MK II /Joyce-Loebl/ type densitometer. Equations and characteristics of the calibration curves were determined - given the knowledge of the integrated areas adequate to protein fractions and the protein concentrations - according to the principle of least squares, using Sharp II. computer. In the case of the studied proteins we found close positive correlation between the concentration and the integrated area values which are the index numbers of the intensity of fractions /Fig. 4., 5./.

Performing determination from a sodium caseinate-meat mixture, using 9,4 % gel concentration and urea solution as medium, the regression constant was between 0,981-0,999 and relative deviation was less than 10 % /Table 1./. When determining soya protein in similar circumstances, regression constants varied from 0,916 to 0,999, relative deviation was under 15 % /Table 2./. The calibration curve has to be made always together with the samples in the case of the determination of both proteins because they may differ in different measurings.

Using the above outlined method, we may gain linear correlation between the concentration and the densitometrically measured intensity values when applying 24-80 µg-s of milk or soya protein. That is to say considering the dilutions taking part in the course of dissolving the samples, there is a possibility for the quantitative determination of milk and soya proteins in meat when being in above 0,4 % concentration, although an amount of 0,2 % milk or soya protein is already detectable.

DETECTION OF MILK PROTEIN FIG.1.

1-BEEF, 2-HEAT TREATED BEEF, 3-BEEF MIXED WITH SODIUM CASEINATE, 4-HEAT TREATED 3, 5-SODIUM CASEINATE

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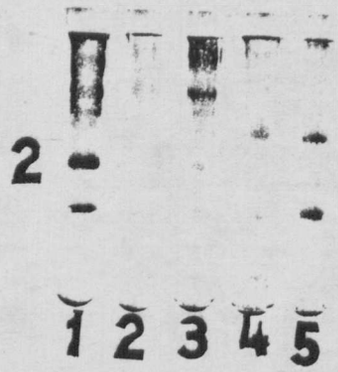


FIG. 2.
ELECTROPHORETIC PATTERN OF PROMINE-D

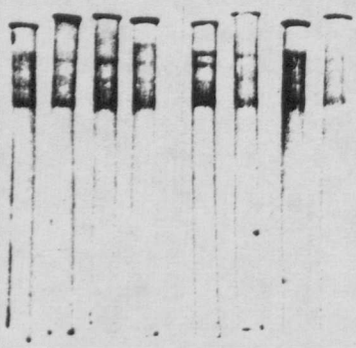

FIG. 3.
ELECTROPHORETIC PATTERN OF DIFFERENT SOYA PRODUCTS

FIG 4
STANDARD CURVE FOR QUANTITATIVE DETECTION OF SODIUM CASEINATE

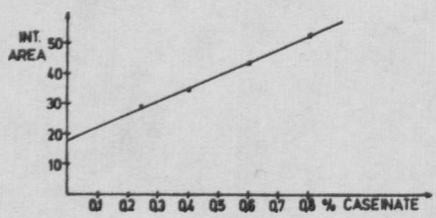


FIG. 5.
STANDARD CURVE FOR QUANTITATIVE DETECTION OF SOYA PROTEIN

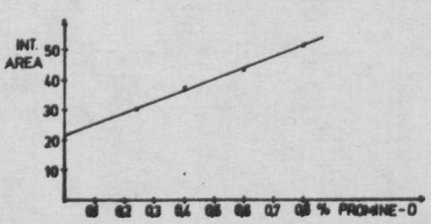

TABLE 1.

CHARACTERISTIC DATA OF THE STANDARD CURVES FOR QUANTITATIVE DETECTION OF SODIUM CASEINATE

| a | b | r | Sxy % |
|---|---|---|---|
| 58,28 | 23,31 | 0,993 | 4,94 |
| 46,14 | 26,07 | 0,997 | 3,10 |
| 43,17 | 18,23 | 0,999 | 1,23 |
| 41,79 | 13,36 | 0,999 | 0,61 |
| 42,86 | 31,86 | 0,993 | 4,56 |
| 40,67 | 25,93 | 0,987 | 4,8 |
| 58,34 | 39,91 | 0,981 | 6,5 |
| 79,57 | 9,02 | 0,994 | 5,23 |
| 67,94 | 17,15 | 0,988 | 6,59 |
| 53,25 | 22,54 | 0,991 | 4,69 |
| 34,13 | 28,68 | 0,998 | 1,86 |
| 32,56 | 43,64 | 0,982 | 3,08 |

TABLE 2.

CHARACTERISTIC DATA OF THE STANDARD CURVES FOR QUANTITATIVE DETECTION OF SOYA PROTEIN

| a | b | r | Sxy % |
|---|---|---|---|
| 22,75 | 22,17 | 0,982 | 4,22 |
| 31,61 | 14,98 | 0,972 | 7,9 |
| 54,12 | 12,50 | 0,994 | 4,36 |
| 30,08 | 40,24 | 0,993 | 3,31 |
| 53,57 | 18,83 | 0,976 | 8,2 |
| 36,42 | 21,66 | 0,999 | 1,36 |
| 59,58 | 1,93 | 0,998 | 3,35 |
| 44,76 | 16,59 | 0,993 | 4,38 |
| 58,13 | 13,86 | 0,991 | 5,22 |
| 81,43 | 17,22 | 0,966 | 11,01 |
| 41,70 | 20,48 | 0,914 | 13,18 |
| 38,89 | 5,78 | 0,998 | 3,91 |
| 23,89 | 11,62 | 0,917 | 14,88 |