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Electrophoretic detection of non-meat proteins in meat products

by

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1. INTRODUCTION

The application of extraneous proteins in meat products as substitutes for part of the meat protein is tied down to certain limited quantities in some countries, but entirely forbidden in others. The detection difficulties experienced with these proteins, especially in highly heated products, and the lack of reliable quantitative methods of determination in recent years has led to a much wider use in the meat industry than advocated by the legislators. In the U.S.A. this problem has been solved by adding a small quantity of a foreign, easily recognizable, component as a "marker" (titandioxide in soy protein). Other countries hesitate to take such measures, which emphasizes the necessity of developing methods permitting detection and -subsequently- quantitative estimation of these additives. The procedures used until lately, which are based on the presence of more or less constant amounts of characteristic non-protein substances (such as lactose and calcium in non-fat dry milk) have become useless, because nowadays several preparations are commercially available that are cleared of these "tracers".

Methods that can be expected to be successful in these circumstances can only be based on characteristic properties of specific protein components. For the detection of casein in meat products advantage has already been gained from its relatively high content of protein-bound phosphor (6, 10, 11) and from the fact that this protein is incoagulable by heat and has moreover a low isoelectric point (2, 8). For the rest the application of serological and electrophoretic methods is obvious. Among the serological procedures the precipitation reactions (9, 12) and the technique of the indirect haemagglutination (3, 4, 5) seem to give good results. Especially the latter procedure is highly sensitive, but on the other hand very time-consuming, requires a lot of work to be done beforehand and demands great skill from the investigator. The only electrophoretic method hitherto published (1) does not lead to results quickly either. The sensitivity of both techniques will diminish with increasing heating temperature and - time of the product during its manufacture.

The method of detection to be described in this paper is based on a rigorous desintegration of the protein coagulum of the meat product sample under consideration by treating it with concentrated urea and mercaptoethanol, followed by a separation of the protein complex in its constituents by means of urea-starch gel electrophoresis.

The detection limits of caseinates and isolated soy protein in luncheon meats, sterilized at 120°C, appeared to be 0.5 and 1.0% respectively (on whole sample basis). The results can be obtained within 24 hours after receipt of the sample.

2. METHODS

2.1. Starch gel electrophoresis

Apparatus and experimental procedure were as specified by Wake & Baldwin (12), except for the preparation of the gel which contained 0,036 molar mercaptoethanol and was prepared otherwise, viz. according to the recipe given by Schmidt (7). The electrophoretic run took place in a cold room (10°C) for 16 hours at a voltage gradient of about 4 V/cm. Photographs were made in reflected light using a Contaflex 50 mm camera and an Agfa Agepe FF orthochromatic film. Printing is done on Agfa Brovira special BS I paper.

2.2. Sample pretreatment

10 g of the comminuted and homogenized sample are heated with 100 ml of a 0.1 m acetate buffer, pH 4.2, for 1 hour under occasional stirring. Then the floating fat layer is decanted and the remaining mixture is centrifuged for 5 minutes at 3000 r.p.m. The supernatant is discarded. The residue is washed once with water and then dried in a stream of warm air. To 1 g of the material obtained 9 ml of 8 m urea, 1 ml of 0.76 m tris-citrate buffer and 5 drops of mercaptoethanol (up to a concentration of 0.2 molar in the mixture) are added, after which the mixture is dispersed by agitating it for 30 seconds with a high speed homogenizer (Ultraturrax). After 2 hours at 10°C the mixture is applied to the gel and the electrophoresis is started.

3. RESULTS AND DISCUSSION

With the technique described above electropherograms were obtained of several non-meat proteins that could be used in meat products (hen's egg white, blood plasma, casein, soy protein, wheat gluten protein, fish protein).

Casein preparations and isolated soy protein (promine-D^{R}) produced the most pronounced electrophoretic patterns. As these preparations are also the ones that are most widely applied, in the first instance we confined ourselves to studying the detectability of these two proteins in meat products. Luncheon meats of known composition were made in the sausage kitchen of the meat department. The electrophoretic pattern of the samples prepared with promine-D and caseinate are significantly different from those of blank samples (these samples were of identical composition except for the extraneous protein) (photographs 1 and 2).

The method of Wake & Baldwin is followed in comparing the band positions in the electropherograms with some marked band, the distance of which from the application spot is arbitrarily set at 1.00. The location of the band can then be indicated by a number. As a reference we used a mobile narrow meat protein band, which was always visible, even in highly heated products. Fig. I shows that the band 0.74 is typical for soy protein in the luncheon meat patterns. Its location corresponds with that of a relatively strong band in the pattern of promine-D itself which contains about 10 bands. In Fig. I e, f and g another diffuse band at 0.83 can be discerned. Other promine-D bands are not visible, unless its amount in the meat product sample is very high.

For products with caseinates the characteristic bands at 0.91 and 0.83 are conclusive, the former mostly being the strongest. A third diffuse band at about 0.58 is visible with higher casein concentrations.

Remarkably enough, these bands do not coincide with the characteristic casein a_s- and β- bands (at the relative positions 0.84 and 0.62 respectively) in the pattern of the commercial preparations (cf. Fig. II). The cause of this phenomenon is unknown to us.

Concentrations of 0.5% of casein and 1.0% of promine-D could just be detected in luncheon meats heated at 120°C at F_o = 3.0.

Higher processing temperatures and longer heating periods turned out to influence the detectability unfavourably (Fig. I, II and III). On the other hand, the patterns were not noticeably affected by the presence of polyphosphates (0.3% as P_2O_5) and (starch 3.5%) and by the relative proportions of muscular and connective tissue proteins.

Because of the susceptibility of its sensitivity to temperature and time of heating, the method can never be used for an exact quantitative determination of extraneous proteins in meat products of unknown processing history. It must be pointed out that this virtually holds for all methods based on specific protein properties, including the Thalacker method (which suffers from interference due to hydrolysis of the protein bound phosphor during the heat treatment of the casein containing products). Hydrogen bond interaction and, to a certain extent, interactions due to hydrophobic bonding can be cancelled by urea and disulphide bridges can be split by mercaptoethanol.

However, this disaggregation into individual protein molecules is far from complete. The ever proceeding entanglement of the protein coils, when after their denaturation the temperature is still rising or kept at a certain (elevated) level for some time, results in an aggregate of ever increasing stability and inaccessability due to growing numbers of Coulomb interactions and the formation of covalent bonds (e.g. intermolecular thioether bridges) being difficult to break down by means that leave the polypeptide chains intact.

Nevertheless the method is very promising, not only for the detection of the possible presence of foreign proteins in meat products, but also, of course, for the characterization of meat substitutes as such.

4. SUMMARY

A detection method is described for casein and soy protein in heatprocessed meat products by means of urea-starch gel electrophoresis. In luncheon meats heated at 120°C the detection limits for casein and soy protein are 0.5 and 1.0% respectively (on whole sample basis). Results can be obtained within 24 hours.

RESUMÉ

Une méthode est décrite pour la détection de la caséine et de la protéine de soya par électrophorèse en gel d'amidon avec l'urée. En saucissons, échauffés à 120°C les limites de détection sont 0.5 et 1.0% pour la caséine et la protéine de soya respectivement (en échantillon total). Les résultats peuvent être obtenus dans les 24 heures.

ZUSAMMENFASSUNG

Es wird eine Nachweismethode für Kasein und Sojaeiweiss mittels Ureum-Stärkegelelektrophorese beschrieben. In bis auf 120°C erhitzten Luncheonmeats sind die Nachweisgrenze für Kasein und Sojaeiweiss 0.5 resp. 1.0% (bezogen auf der ganzen Probe). Resultate sind innerhalb 24 Stunden zu erhalten.

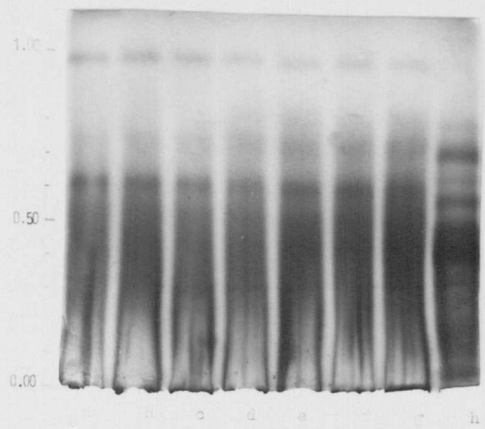

Fig. I

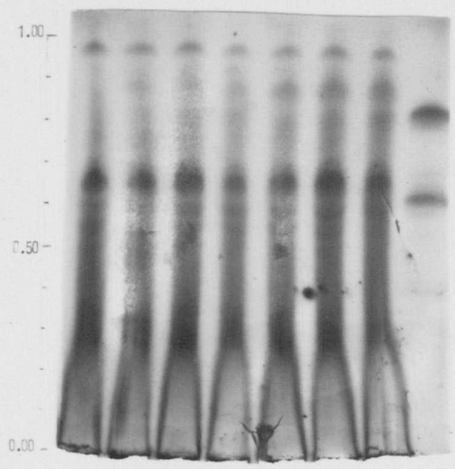

Fig. II

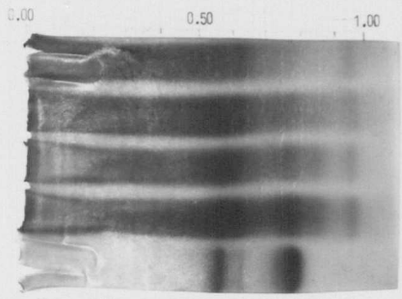

Fig. III

Fig. I. Luncheon meats prepared with isolated soy-protein (promine-D^{R}) and heated at 105°C, sterilization value F_o= 0.2
a. 0% promine-D, no polyphosphates added, moderate collagen content
b. 1.0% promine-D, for the rest as a
c. 1.0% promine-D, 0.3% polyphosphates added, moderate collagen content
d. as c, but high collagen content
e. 2.0% promine-D, for the rest as b
f. 2.0% promine-D, for the rest as c
g. 2.0% promine-D, for the rest as d
h. promine-D; 1.0% in tris-buffered urea solution

Fig. II. Luncheon meats prepared with sodium caseinate, heated at 105°C, sterilization value F_o= 0.2
a. 0% caseinate, no polyphosphates added, moderate collagen content
b. 0.5% caseinate, for the rest as a
c. 0.5% caseinate, 0.3% polyphosphates added; moderate collagen content
d. as c, but high collagen content
e. 1.0% caseinate, for the rest as b
f. 1.0% caseinate, for the rest as c
g. 1.0% caseinate, for the rest as d
h. sodium caseinate; 0.4% in tris-buffered urea solution

Fig. III. Luncheon meats prepared with and without 2.5% sodium caseinate and heated at 110°C at different times with and without 3.5% starch
a. 0% caseinate, starch added, F_o-value 2.0
b. 2.5% caseinate, for the rest as a
c. as b, but F_o= 0.4
d. as c, but without starch
e. sodium caseinate o.4% in tris-buffered urea solution

5. REFERENCES

1. Baal, J.P.W. van & J.N. Leget, Nachweis von Casein und Natriumcaseinat in Fleischprodukten
Z. Lebensm.-Untersuch. u. -Forsch. 127 (1965) 263-268

2. Fleischmann, O., Nachweis und Bestimmung von Milcheiweiss in Fleischerzeugnissen
Die Fleischwirtschaft 44 (1964) 985-988

3. Herrmann, C. Über den Nachweis von hitzedenaturiertem Hühnereiklar in Fleischwaren mit Hilfe der indirekten Hämagglutination
Inaugural Diss. München 1963

4. Herrmann, C., Zum quantitativen Nachweis von aufgeschlossenem Milcheiweiss in hocherhitzten Fleischwaren mit Hilfe der indirekten Hämagglutination
XIth European Meeting of Meat Research Workers, Beograd 1965

5. Kotter, L., C. Herrmann & J. Corsico, Zum quantitativen Nachweis von aufgeschlossenem Milcheiweiss in hocherhitzten Fleischwaren mit Hilfe der indirekten Hämagglutination
Z. Lebensm.-Untersuch. u. -Forsch. 133 (1966) 15-22

6. Kutscher, W., W. Nagel & W. Pfaff, Nachweis und quantitative Bestimmung von Milcheiweiss in Fleischerzeugnissen
Z. Lebensm.-Untersuch. u. -Forsch. 115 (1961) 117

7. Schmidt, D.G., Starch-gel electrophoresis of K-casein
Biochim. Biophys. Acta 90 (1964) 411-414

8. Schneck, A. & M. Ziegler, der Nachweis von aufgeschlossenem Milcheiweiss in Wurstwaren
Vorratspflege u. Lebensmittelforschg. 1 (1938) 494-499

9. Sinell, H.-J., Verarbeitung von Hühnereiweiss in Brühwurst
Berl. u. Münch. Tierärztl. Wchschr. 75 (1962) 48-51

10. Thalacker, R., Bestimmung von Milcheiweiss in Fleischerzeugnissen
Dtsch. Lebensm. Rundsch. 59 (1963) 111-113

11. Thalacker, R., Vereinfachtes Verfahren für die Ermittlung des Phosphoproteid-Phosphors in Fleischerzeugnissen (Bestimmung von Milcheiweiss)
Lebensm. chem. u. ger. Chemie 20 (1966) 45-49

12. Wake, R.G. & R.L. Baldwin, Analysis of casein fractions by zone electrophoresis in concentrated urea
Biochim. Biophys. Acta 47 (1961) 225-239

13. Wyler, O. & J.-J. Siegrist, Einfache, empfindliche Nachweismethode für fleischfremde tierische Eiweissarten
Mitt. Geb. Lebensm. u. Hyg. 56 (1965) 299-303

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