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Immunelectrophoretic detection of denatured milk protein in heated meat products and studies on its quantitative evaluation.

By H. J. Sinell and Renate Kluge-Wilm.

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IMMUNOELECTROPHORETIC DETECTION OF DENATURED MILK PROTEIN IN HEATED MEAT PRODUCTS AND STUDIES ON ITS QUANTITATIVE EVALUATION

(Immunelektrophoretischer Nachweiss von aufgeschlossenem Milcheiweiss in erhitzten Fleischerzeugnissen und Versuche zu einer quantitativen Auswertung)

By H.-J. Sinell and Renate Kluge-Wilm

With 4 illustrations

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Used as additive to meat products, milk protein increases the water bond and improves the fat emulsifying effect of the muscle protein. As a result it enjoys a special popularity, in particular in the preparation of very finely ground products. As is generally known, the use of this additive is permitted in the Federal Republic of Germany only in a few products described in greater detail in the Regulation on Meat and Meat Products (Fleischverordnung) of December 19, 1959, and is limited to an amount of at most 2%. This creates for the food inspection service the problem of a sufficiently reliable quantitative detection. This problem has been repeatedly reported on and discussed in literature. Up till now chemical or biochemical (Kutscher, Nagel, Pfaff, 1961; Thalacker, 1963; van Baal and Leget, 1965) and a few serological methods (cf. below) have been described. In each one of the publications dealing with this problem our attention is drawn in particular to the difficulties and uncertainties of the quantitative detection. This problem is also dealt with in this article by using the immunoelectrophoretic detection as an example.

Serological detection methods for milk protein have been developed on the basis of the diffusion method according to Ouchterlony, the immunohistochemistry, the indirect haemagglutination, and the immunoelectrophoresis.

Hanson (1964) developed an immunological technique for the qualitative determination of milk protein in food, whereby he used blood plasma as a model. With the aid of the "comparative immunoelectrophoresis" the reaction between electrophoretically unripped cattle colostrum and the corresponding antiserum was modified here by the addition of a third component to the agar-gel. This third component consisted of the investigation material to be tested. When milk protein was present the precipitate bands in the gel were changed and deflected in a characteristic manner. The comparative immunophoresis is supposedly ~~skill~~ able to make an addition of dry milk up to 0.1% to the investigation material still visible.

The serological technique was also used already P. 803 by different parties for the investigation of meat products. Wyler and Siegrist (1965) succeeded with the aid of the diffusion method according to Ouchterlony to carry out a qualitative detection of denatured milk protein and skimmed milk powder up to an addition of 0.1%. This method was based on the serological reaction in the agar-gel between a sodium caseinate serum and extracts from material to which denatured milk protein or skim-milk powder had been added. The authors assumed that from the strength of the precipitation line no conclusion can be drawn with regard to the concentration of milk protein in the meat product. The

effect of the manufacturing temperature of the meat product on the serological reaction was not investigated.

Holpert (1965) carried out a qualitative detection of denatured milk protein in a histochemical manner: frozen sections of milk protein-containing meat products were coloured with fluorescence-marked antibodies. Under the fluorescence microscope the respective AG-AK complexes showed a dot-shaped fluorescence appearance.

Chr. Herrmann (1965) as well as Kotter, Herrmann and Corsico (1966) described the quantitative detection of denatured milk protein in highly heated meat products on the basis of the indirect haemagglutination. With the aid of anti— milk protein sera it was possible to detect quantitatively an addition of denatured milk protein of 0.1% and more in material heated up to 115° C.

Recently Degenkolb and Hingerle (1967) have also dealt in a very thorough study with, among other things, the detection of milk protein in meat products. Their detailed data on the technical carrying out of the investigations orient themselves to the precipitation and the haemagglutination and are essentially adjusted to the interests of practical routine diagnostics. Correspondingly, in Degenkolb and Hingerle data on the quantitative evaluation of results are found only to the extent as they concern the

ascertainment of transgressions of threshold values.

Fromm (1967) recently proposed a modification of the latex test. According to the author, the method is suitable for an approximate quantitative evaluation.

An attempt was made by Kluge-Wilm (1967) to apply the above-mentioned Hanson technique of the immunoelectrophoresis also in the investigation of meat products in a modified form. It was again found that the milk protein can readily be detected by means of serological methods. At the same time, however, here too certain difficulties arose in the quantitative interpretation of the results. This suggested the carrying out of further experiments which will be reported on in this article.

Material and Method

Antisera

Antisera were obtained by the immunization of rabbits with a commercial preparation of denatured milk protein. At a minimum age of 6 months the animals had an average weight of 3.5 kg and were clinically healthy. At the beginning of each immunization a test precipitation took place between the normal serum of the experimental animal and the antigen, as well as between the normal sera and the muscle extracts from cattle, horse, and swine in order to exclude from the

experiments unspecifically reacting animals. Prior to the immunization the antigen was heated for 15 minutes at 100° C and immediately cooled off under flowing water and lyophilized (dry antigen). P. 804

The mode of immunization used included the combined application of antigen with Freund's adjuvant. Used was Bacto-Adjuvant Complete Freund (Difco). This adjuvant is an emulsion consisting of paraffin oil with a stabilizer (lanoline derivative) to which was added a suspension of mycobacterium butyricum. At first, each animal was administered intracutaneously 2 ml of a suspension consisting of 4 mg dry antigen, 1 ml phys. NaCl, and 1 ml Freund adjuvant. After the expiration of 2 weeks a three-day immunization followed, whereby on the first day 10 mg of antigen were administered subcutaneously, while on the second and on the third day 10 mg of antigen were administered intravenously, always in 3 ml phys. NaCl. After another eight days the titers were checked and the rabbits were bled to death from the vena jugularis. The titer determination of the antisera took place by means of the precipitation method in a small tube according to Uhlenhuth in the α-procedure (Dean and Webb, 1926). For this purpose the undiluted antiserum from the rabbit which was to be investigated was carefully covered in a small narrow tube with the antigens adjusted and geometrically diluted according to the Biuret

protein content to 0.1 g%. The reaction was read off against a black, obliquely-placed background. As titer of an antiserum we regarded that dilution of the corresponding antigen which still barely produced, after a reaction period of 20 minutes, a circular precipitate in the tube. The homologous titer refers to the antigen used in the preparation of the antiserum which was to be tested.

Investigation Material

As investigation material we used sample sausages with varying additions of milk protein. After their manufacture these sausages were heated at different temperatures. The sausages were prepared from lean pork used for thick sausages. It was taken from the current production of a local factory of meat products without additives as control, and with additives of 0.25, 0.5, 1.0, 2.0 and 4.0% milk protein for the test. The lean pork and the milk protein were mixed carefully by hand, pressed with the aid of a hand-gun into dried guts, marked, smoked, and boiled for 20 minutes at 85^{0} C. Before the addition of milk protein the lean pork for the thick sausage had a water content of 56.57%. Each charge with equal addition of milk protein was once again subdivided into individual portions of 50 g, heat-sealed in plastic bags, and stored at -20^{o} C.

One part of the sample sausages with the milk-protein

concentrations of from 0% to 4% was later autoclaved in beaker flasks sealed with metal foil for 30 minutes at 115° C, 120° C, or 125° C and then immediately stored until the further investigation at a temperature of -20° C.

To prepare the extract, 5 g of the investigation material, finely ground in the mixer, were mixed with 5 ml of boiling phys. NaCl, vigorously shaken, and extracted for 24 hours at room temperature. The mixture was then centrifuged (30 minutes at 5000 r.p.m.) and the excess filtered off.

Immunoelectrophoresis

In the present experiments we used the immunoelectrophoresis equipment 6800 A of the firm LKB Stockholm according to the pertinent operating instructions. The apparatus makes possible on the slide an electrophoretic analysis according to the micromethod introduced by Scheidegger (1955). On slides covered evenly with a 1% buffered agar, two round antigen basins with a diameter of 1mm and a 1 mm-wide and 6.5 mm-long groove between them were punched out P. 805 per slide with the aid of a special punching form. The grooves served for receiving the antiserum. The two antigen basins were each filled with from 1 to 2 µl of the substance to be investigated; they were then separated within one hour in the electric field (250 v, 50 mA).

Immediately after the separation 0.04 ml of antiserum were placed in the preformed groove. The antiserum diffused within 20 hours toward the electrophoretically separated investigation material (= antigenes) located on both sides of the antiserum groove. Any excess non-precipitated protein was then removed by dipping the slide for 24 hours into physiol. NaCl and distilled water. The AG-AK reaction became visible through the formation of opalizing precipitates in the agar-gel. For clarification these were then dyed with amido-black, after the preparations were completely dry.

The material investigated with the aid of the immuno-electrophoresis was then evaluated according to the localization, the course and the strength of the resulting precipitation bonds. The identification and designation of the discovered precipitates is based on the designations of the milk proteins in the works of Gugler, Bein and von Muralt (1959).

For further methodical details the reader is referred to the work of Kluge-Wilm (1967).

Results

Antisera

The production of a high-quality antiserum with a very high homologous titer and sufficient specificity was regarded as the yardstick for the success of the immunization with milk protein. The antisera 6 x 13 and 87 prepared with altogether 34 mg of antigen heated to 100° C and a simultaneous application of Freund's adjuvant had a homologous titer of 1 : 256,000. The titers against unheated antigen and another commercial preparation with denatured milk protein also amounted to 1 : 256,000 in both cases. Against raw and autoclaved skimmed milk the titer amounted to 1 : 128,000. The negative serological reactions with normal sera and muscle extracts from cattle, horse, and swine indicated a sufficient specificity of the antisera. The anti-milk protein sera were also titrated out against acid and rennet-casein solutions. The titer used for precipitating the casein preparations was always by one to two dilution stages lower than that used for precipitating the homologous antigen.

Sausage, normal preparation, not autoclaved

The extracts of the sample sausages formed in all tested milk protein concentrations (0.25 to 4.0%) -- both

in autoclaved as well as in non-autoclaved material -- on the anode side in the casein region precipitates the strength of which depended on the amount of the milk-protein addition and to some extent on the preceding heating temperature (Fig. 1). The negative controls, on the other hand -- sausage without milk-protein addition -- did not show any precipitation bonds with the anti-milk protein serum.

At a milk-protein addition of 4% (preparation 326) a strongly developed, evenly curved precipitation arc was formed in the casein range. Its cathodic end extended almost to the antigen basin and appeared fuzzed. The anodic end of the arc consisted of a powerfull, short, strongly curved flank and a fine, long, and quite flat precipitation line, running parallel to the antiserum groove. This precipitation line was split at the ends. This precipitate P. 806 can be related only to the milk-protein addition, since the negative control (sausage without milk protein) did not produce any visible precipitates with the same antiserum. With a decreasing milk-protein concentration the precipitates became smaller. Finally, there remained only the central part which, barely visible, is located in the casein range. Already below 2% the splitting up of the flanks could not be observed any more. As shown in Fig. 1, the varying intensity and configuration of the precipitates permits an easy differentiation of the milk-protein additions to the sausage in the mentioned degrees of concentration.

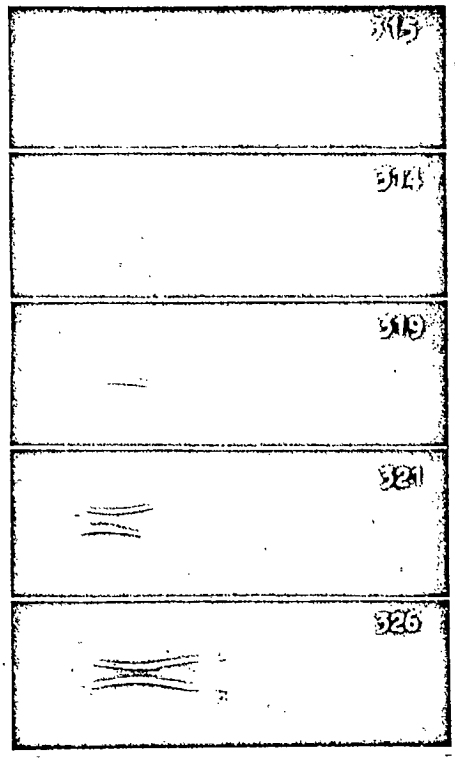

Fig. 1. Reaction between extracts from fresh sausages with varying milk-protein additions and anti-milk protein serum.

315 = 0.25% milk-protein addition
314 = 0.5 % milk-protein addition
319 = 1.0 % milk-protein addition
321 = 2.0 % milk-protein addition
326 = 4.0 % milk-protein addition

Autoclaved Sausages

After a temperature reaction of 115° C as well as of 120° C the sample sausages showed distinct differences in the formation of precipitates in the concentrations of 0.25, 0.5, 1.0, 2.0, and 4.0% milk-protein addition. As was already observed in the case of the non-autoclaved sausages, here, too, the centre of the precipitation arcs lay in the

casein range. If the additions were small, a short, practically unbent precipitate was formed. At higher concentrations the latter appeared bent. Anodically as well as cathodically it extended far into the β-lactoglobuline and α-lactalalbumin range. In comparing the autoclaved with the non-autoclaved material it becomes evident that the extracts from the material autoclaved at 115° C (Fig. 2) and at 120° C (Fig. 3) produced longer and more strongly dyed precipitates than equal milk-protein concentrations of the non-autoclaved material (Fig. 1).

Sample sausages which were autoclaved for 30 minutes at 125° C formed with the corresponding antiserum in all milk-protein concentrations visible precipitation bonds in the casein range (Fig. 4). However, in contrast to the less heated material described above, these were only indistinctly outlined and somewhat diffusely tinted in the concentration range of 0.25, 0.5, and 1.0%. Concentration-dependent gradations could practically not be determined any more in this range. Only with 2- and 4%-additions were clearly tinted, sharper outlined precipitates formed which, in contrast to the already described experimental groups, extended farther. Only the anodic end of the arc terminated in a slight up-turning, while the cathodic end continued unbent, parallel to the antiserum groove in the vicinity of the antigen basin (preparation 1024, 1027).

P. 807

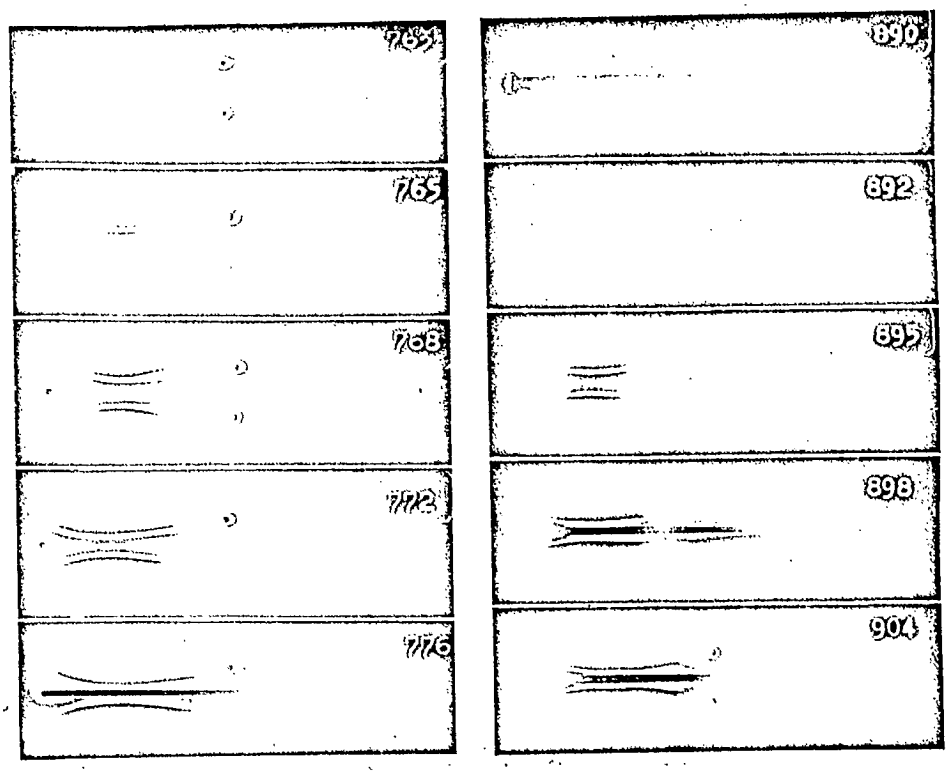

Fig. 2. Reaction between extracts from canned sausages (115° C) with varying milk-protein additions and anti-milk protein serum.

763 = 0.25% milk-protein addition
765 = 0.5 % milk-protein addition
768 = 1.0 % milk-protein addition
772 = 2.0 % milk-protein addition
776 = 4.0 % milk-protein addition

Fig. 3. Reaction between extracts from canned causages (120° C) with varying milk protein additions and anti-milk protein serum.

890 = milk-protein addition
892 = milk-protein addition
895 = milk-protein addition
898 = milk-protein addition
904 = milk-protein addition

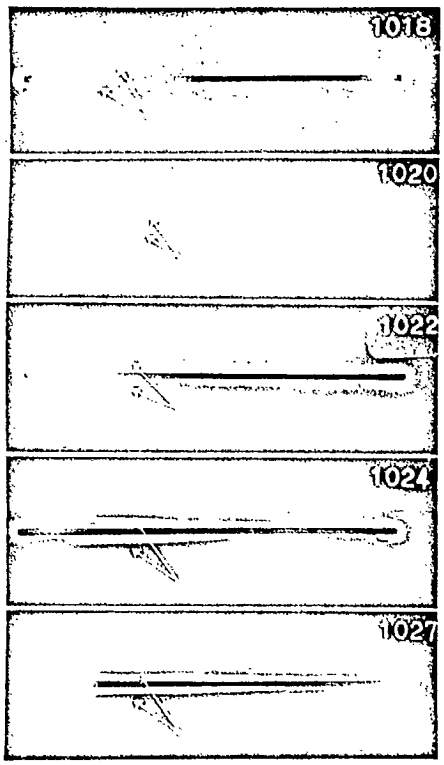

Fig. 4. Reaction between extracts from canned sausages (125° C) with varying milk-protein additions and anti-milk protein serum.

1018 = 0.25% milk-protein addition
1020 = 0.5 % milk-protein addition
1022 = 1.0 % milk-protein addition
1024 = 2.0 % milk-protein addition
1027 = 4.0 % milk-protein addition

The concentrations of 2% and 4% could also be distinguished from each other only with difficulties. Thus, while the immunoelectrophoretic investigation of the material autoclaved at 125° C made possible a qualitative detection of milk protein in all employed milk-protein concentrations, the quantitative differentiation on the basis of the formation of precipitates, however, encountered difficulties. All that could be distinguished with some degree of certainty was whether the milk-protein addition to the sausage material was higher or lower than 1%.

Titration Experiments

The concentration-dependent absence of individual reactions manifested in the above experiments induced us to check the level of the immunoelectrophoretic titer in the different extracts. Under standardized experimental conditions we expected to find in the initial material at best a direct dependence between extract titer and milk protein concentration. P. 808

For this purpose geometric dilutions of the extracts adjusted according to the Buiret-protein content were immunoelectrophoretically separated and made to react with the corresponding anti-milk protein sera. The dilution which still showed visible precipitate bonds in the casein range was designated as "titer." The results are shown in the table. As was to be expected, the milk-protein concentrations of the initial material and the titer manifested a close correlation with each other. However, the table also shows that there does not exist a strict linear relationship. Higher milk-protein concentrations in the investigation material led to still higher titers in the extracts. Of importance for the subsequent interpretation of this phenomenon is the fact that the "titer" is being derived from the Biuret protein content which, as will be discussed later, needs by no means be proportional to the antigen-protein content.

Table

Terminal titer of the extracts from sample sausages with milk-protein additions of from 0.25 to 4.0% heated at different temperatures

Temperature	Milk-protein addition to the sausage meat				
	0.25%	0.5%	1.0%	2.0%	4.0%
not autoclaved	1:200	1:370	1:400	1:1250	1:5880
115° C	1:240	1:470	1:840	1:5880	1:9090
120° C	1: 90	1:180	1:310	1:1100	1:4000
125° C	1: 40	1: 80	1: 80	1: 260	1: 890

In the compilation it seems further noteworthy that the level of the terminal titer depends not only on the milk-protein concentration in the initial material, but also on the degree of the preceding heating. While the decline of the serological reactivity in the material heated at 120° C is not yet very conspicuous, after the heating at 125° C it is very pronounced. The titers are several times lower as compared to the material heated at a lower temperature with the same milk-protein concentrations.

In the reproduction of the qualitative findings we already pointed out the conspicuously weaker reactions in the lower-heated meat products as compared to the higher-heated goods. In the above table this observation also

finds its qualitative expression. Here the titers lie, in part, considerably lower than in the autoclaved material.

Discussion P. 809

Generally, no difficulties are encountered in obtaineing sera with sufficiently high titers against denatured milk protein, especially when an adjuvant is used along with it (cf. Ring, 1964; Holpert, 1965; Kotter and co-workers, 1966). However, the specificity of such sera leaves much to be desired. This considerably restricts its value for investigations within the framework of the food control. On the basis of the results of earlier immunizations with different milk-protein fractions it had been assumed that the commercial preparations used for immunization contain, in addition to the decomposition stages of casein, also very small admixtures of various whey proteins (Kluge-Wilm, 1967). When using unheated milk protein these impurities are apparently responsible for species-homologous cross reactions of these sera with serum and muscle proteins. This disadvantage was avoided by a heating of the antigen to boiling temperatures. During this process the milk-unspecific whey proteins are inactivated, the immuno-chemical polyvalence of the unpretreated material is eliminated. Because of their essentially higher heat stability the casein decomposition products do not suffer any loss of their

serological activity and the thusly obtained sera distinguish themselves by a higher specificity, i.e. they are milk-specific. Here we are not dealing with pure anti-casein sera, such as were prepared already by Grätz (1911) by immunization with boiled milk (so-called "coctolacto-sera"). This is rather a case specifically against the decomposition products of casein present in the denatured milk protein. As shown by orientating investigations (11), these sera react with casein, but only in a low temperature range.

In the treatment of the present problem the immuno-electrophoresis has proved to be an especially valuable method. It is just as sensitive as any other serological method. On the other hand, it makes possible a differentiation of the results such as is not found in any other routine-like technique today. This encouraged us to risk also an attempt at a quantitative evaluation.

In previous investigations (11) it was already found that pure solutions of denatured milk protein produced in the immunoelectrophoresis precipitation bonds the intensity and configuration of which were strictly dependent on the antigen concentration. In the present experiments on sample sausages with gradated milk-protein additions basically the same behaviour could be observed. Beyond that we noticed

many things which shall be especially mentioned here because of their general importance for the quantitative evaluation of serological findings in the diagnostics of foreign proteins.

Thus, first of all, it is worth noting that while the milk-protein concentrations in the initial material were equal, the precipitation bonds developed varyingly, depending on the temperature to which the material had been heated. In this connection it was especially remarkable that extracts from autoclaved material (120° C and 115° C) formed stronger and more prolonged bonds than were formed in fresh goods, heated at a low temperature, with an equivalent milk-protein addition. Such an observation is completely contradictory e.g. to the bahaviour of muscle or serum proteins of which we know that they react the weaker, the higher they have been heated. P. 810

Two explanations may be considered for this behaviour. It is a well-known fact that milk proteins and in particular the casein show an especially high heat stability. According to Hanson (1961) casein can endure a heating at 120° C for 15 minutes without any detectable changes in its specific immunoelectrophoretic properties. Among the muscle proteins, on the other hand, even the somewhat more stable sarcoplasma proteins are irreversibly denatured already at far lower temperatures. For this reason it may be assumed that an

extract from highly heated sausage material will contain at least a relatively higher proportion of milk-protein bodies than a similar extract from material of the same composition, but heated only to a low temperature. The muscle-milk protein ratio in an extract thus will shift in favour of the milk protein, and this the more so the higher the original material has been heated. The extracts for the immunoelectrophoresis are not evaluated according to their antigen nitrogen, but according to the (gross) protein content determinable in the Biuret reaction. An extract from a higher heated material adjusted to 0.1 g% Biuret protein content thus also contains absolutely a higher milk-protein portion than a correspondingly adjusted extract from a lower heated material.

As a matter of fact, we observed not only varying titers, but also quite variously formed precipitation bonds. Especially conspicuous is the fact that the lines from the highly heated material were prolonged extremely far in the anodic as well as in the cathodic range. It is conceivable that this phenomenon may be traced back to thermally conditioned interactions of the casein with β-lactoglobulin or α-lactalbumin. In literature we find descriptions of complex formations which are formed during the heating of milk or of mixtures of casein and whey proteins above a certain temperature. Such interactions are known of

β-lactoglobulin with total casein (Rose, 1965), with α-casein, with χ-casein (Zittle etc., 1962; Hartmann and Swanson, 1965) as well as with α-lactalbumin (Hunziker and Tarassuk, 1965).

The complex formations go back to reactions of sulfhydryl and disulfide groups which are freed in the α-lactalbumin and β-lactoglobulin and react with one another. Apparently, in casein or its serologically still active breakdown products, too, a far-reaching denaturation has occurred with a temperature of 125° C. While positive reactions still take place, they are, however, considerably weaker than in lower heated material of the same composition. The reactivity of the milk protein has changed to such a degree that a definite assignment of the various reactions to certain concentrations is not possible any more.

The milk-protein share in an extract from unknown investigation material by means of immunoelectrophoresis could possibly be determined with the aid of titration. However, our experiences show that with the immunoelectrophoresis the changes of the titer do not take place concentration-linearly (cf. table). Orientating comparisons with the small tube precipitation confirm this. The linear relationship between antigen concentration and precipitating titer applies only to dilutions of the pure antigen substance, but apparently not when the latter is present in a mixture with other protein bodies. For the practice this means that

P. 811

the specific titer of an antiserum can be regarded as constant magnitude only as long as defined and mutually comparable conditions prevail also in the antigen dilutions. This realization is not exactly new in serology. It leads however to a consequence which considerably aggravates the quantitative detection; for if the titer of a serum is affected by the accompanying protein content not acting as antigen, we cannot base our yardstick for comparison on titer values which were ascertained on pure antigen dilutions.

Such an extrapolation is prohibited also by a further consideration: in a comparison of the titer of an extract from unknown material with the behaviour of pure test dilutions it would be possible to derive a statement on the milk-protein concentration in the investigated doubtful extracts, but not on its level in the original material. In this direction a conclusion by analogy would be permissible only if the barely visible serological reaction (of the titer) depended directly concentration-linearly on the antigen content of the original material. That this is not the case is evidenced by, among other things, the present experiments. Degenkolb and Hingerle (1967) also hint at similar considerations with regard to the reliability of such a quantitative evaluation.

From this discussion it follows that a reliable quantification of findings in the determination of denatured milk protein in meat products by means of immunoelectrophoresis presupposes very careful control investigations. In these controls prescription and treatment conditions of the original material must have been imitated as exactly as possible. The concentration gradations of the added milk protein must lie in the controls within the range that is being investigated, so that the doubtful findings can be interpreted, as it were, by means of a "calibration series".

In this connection the proposal to refer for the standardization of the extraction (3) to the dry mass of the investigation material definitely deserves consideration.

Finally, there is one more objection. However, as a rule, it will be possible to invalidate same. The strength of the serological reaction depends, among other things, on the degree of the preceding heating. Generally, this factor is not known to the person carrying out the investigation. For this reason he must select in the preparation of his control a heating temperature which still guarantees to him the best reactivity of the investigation material (level of titer) possible. According to available experiences such temperature would lie above 100° C and below 115° C.

It may be left undecided whether the considerations elucidated here on the example of the immunoelectrophoretic detection of milk protein, with the demands derived therefrom for every kind of foreign protein addition and for every quantitative serological detection procedure, can be generalized. In so far as the control investigations are concerned this is the case under all circumstances, according to our own experiences. It has already been demanded previously (17), when the problem was defined from a purely qualitative point of view, that in the biological protein differentiation the control must correspond to the investigation material in as many respects as possible. For a quantitative statement the requirements will be at least the same.

The carrying out of such extensive control investigations as are asked for above demand a considerable expenditure in material and labour. On the other hand, such controls need not be started from the beginning in every case. The necessary preparations may also be held in reserve. Lyophilized and stored under vacuum or also in a deepfreeze state they can be stored for many months, presumably even for years, without losing their capacity of reaction. Nevertheless, the entire process remains a complicated procedure which one will not always be able to afford in the daily routine and which will not do complete justice to the strict yeardstick which must be applied to

P. 812

a quantitative method of analysis. It may be assumed that with accurate quantitative immunochemical methods, e.g. with the antibody or antigen nitrogen determination according to Heidelberger and MacPherson (1943), the analytics may be improved. Pertinent investigations will be reported on at the proper time.

Summary

Qualitatively highly active sera can be prepared against denatured milk protein. Traces of not strictly milk-specific whey proteins can be removed from the antigens by heating at 100° C. The strongest antigen effect in milk protein preparations is apparently related to casein breakdown products. Milk-protein additions can readily be detected in sausages by immunoelectrophoresis. The degree of reaction depends on the concentration in the original material and is also quantitatively measurable in autoclaved material. An addition of 0.25% to the original material could readily be detected with certainty. After heating for 20 minutes at 125° C the material was denatured to such an extent that a quantitative evaluation is no longer possible. However, such a high temperature should have no practical import in the manufacture of meat products. Reliable quantitative evaluations of serological reactions are only possible when controls are avialable which correspond to the source material in as many respects as possible.

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