ELISA - A SENSITIVE AND ECONOMICAL TEST FOR DETECTION AND QUANTIFICATION OF NON-MEAT PROTEINS IN HEATED MEAT PRODUCTS

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SUMMARY: In comparison with OUCHTERLONY and IH the ELISA is the most sensitive and most specific test. One of the most important advantages of the ELISA ist that the results can be measured objectively by photometer or computer. Finally ELISA is less expensive in handling and in the use of sera.
The results of all three serological methods are reproducible. ELISA is therfore - independent of the type of modification - the best serological method for the detection and quantification of non-meat proteins in heated meat products.
ELISA is recognized as the preferred method for detecting toxins and anabolic steroids. The present studies have shown that ELISA can also be recommended as a preferred method in food serology.

INTRODUCTION: Food serology applied to the detection of proteins and hence for distinguishing animal species has an almost 100-year history, but serological detection of non-meat protein added to raw, heated are ultra-heat-treated meat products has only been in use in the Federal Republic of Germany for 30 years or so. In the early days it was applied above all to detecting and quantifying additions of milk protein and wheat gluten, while more recently the target has increasingly been the technologically more attractive soya proteins at various degrees of refinement as well as other vegetable proteins.

In the process, it has become clear that serological procedures superior to chemical or physical-chemical methods for the detection of additional non-meat proteins in heated meat products. The choice of reliable serological methods today is between Ouchterlony's double-diffusion test, electrophoresis, indirect haemagglutination and ELISA. The electrophoresis procedure can be regarded as a further development from Ouchterlony's test.

Studies were conducted to evaluate Ouchterlony's test, indirect haemagglutination and ELISA, and how they compare, in terms of practicability, economy, specifity, sensibility and reproducibility.

The investigations were carried out using test sausages heated to 75°C, 116°C and 121°C to which differing amounts of non-meat protein had been added. Control sausages were also prepared and heated to the same temperatures, but with no proteins added.

RESULTS:

Specifity

The specifity of the three tests depends crucially on the quality of the antisera. Cross-reactions with other proteins can be largely eliminated by purifying the sera of non-specific antibodies by affinity chromatography. Since purifying entails a reduction in the antibody titre (dilution), the sera can only be used for the Ouchterlony and IH tests after concentration. Low titres are sufficient for ELISA.

Table 1: Cross-reactions
ELISA
Serum: antibodies against soya protein

	Soya protein	Milk protein	Ovalbumin
Unpurified serum Titre 1:25 600	pos.	1:40 cross-react.	1:80 cross-react.
Purified serum Titre 1:25	pos.	neg.	neg.
Purified serum/conc. Titre 1:100	pos.	neg.	neg.

As it is evident from Table 1, cross-reactions can be eliminated by purifying sera from non-specific antibodies.

Sensitivity

Table 2 shows that ELISA proved to be the most sensitive test. Non-meat protein levels as low as 0.25% can be detected in confidence with this test.

Method	ELISA	IH	Ouchterlony
Non-meat protein	Soya	Soya	Soya
Detection limit	0.25%	0.5%	1.0%
Steps	0.25%	0.5%	1.0%

Reproducibility

All three methods delivered reproducible results. Several measurements on the test samples using the same test, as well as measurements on the same samples using the different tests, gave unambiguous results if the sensitivity of each test is taken into account. Objectivized results are only possible with ELISA. Using a computer an/or photometer, it is possible to draw a standardized calibration curve to evaluate automatically the results. However, evaluations of IH and Ouchterlony results still have to be assessed subjectively, i.e. "by eye".

Practicability and economy

In this area, too, ELISA is superior to the other tests.

Criterion	ELISA	Ouchterlony	IH
Work involved	low	low	high
Time needed	4 hours	12-48 hours	24 hours
Speed of handling samples	high	high	low
Amount of serum required	low	high	very high