

IMMUNOCHEMICAL DETECTION OF ANIMAL SPECIES IN PROCESSED MEAT PRODUCTS

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Background.

The possibility of substitutions of animal species from one to another in meat products leads to the need of reliable and specific methods to identify these species. These methods are useful for public health and for ethical or religious reasons. They are also needed to assess conformity of food products with labelling and practices and to warrant fairness in trades (Méret *et al.*, 1998). Immunochemistry and DNA methods are often the most efficient kind of analyses in this field (Janssen *et al.*, 1998; Bergwerff, 1999). They give sensitive and reliable results. Previous studies have shown that some epitopes of proteins can be recognized by antibodies after drastic treatments, even when DNA is too denatured to be analyzed (programme contract n° R95/05, Aliment Demain 1994).

Objectives.

To detect animal species in processed meat products using the ELISA kits from Tepnel Biosystems (Deeside, UK). Experimental models were processed meat products containing various amounts of meat from different animal species.

Materials and methods

Meat products containing 50% of a mixture of usual ingredients (fat, water, salts, spices, sugars and proteic binders) and 50% of binary mixtures of meat from different animal species were prepared in the CTSCCV experimental processing laboratory. Binary mixtures were composed of turkey and beef, pork and mutton, chicken and turkey; percentages of animal species ranged from 0% to 50%. Each meat product was prepared according to three different procedures: raw, pasteurized (70°C for 2 h) or sterilized (115°C for 70 min). After processing, the cans were kept at -20°C until protein extraction. Immunodetection of beef, pork, mutton, poultry, turkey and chicken were performed using ELISA kits from Tepnel Biosystems according to the manufacturer's instructions.

Results and discussions

Results of immunodetection of pork were reported in table I. The threshold value was set at the mean of absorbance values of the negative controls x 2.5. It was shown that 0.5% pork was detectable in all products, even when sterilized. Mutton was detected in raw products containing 0.5% mutton, and in pasteurized and sterilized products containing 5% mutton (table IV). The threshold value was 0.088.

Results presented in table II showed that beef was identified respectively in raw (0.5%), pasteurized (0.5%), and sterilized (5%) products.

Poultry was detectable in raw (0.05%), pasteurized (0.5%), and sterilized (5%) products (table IV). The threshold value was 0.112. Results of immunodetection of turkey in products containing chicken and/or turkey were reported in table III. The threshold was set at 0.200 because the mean of absorbance of the negative controls x 3 was inferior to 0.200. Turkey was identified in raw (0.5%) and pasteurized (5%) products, but not in sterilized products. Similar results were obtained when turkey was detected in the products containing beef and/or turkey (table IV). Similar results were also obtained for the detection of chicken in products containing chicken and/or turkey (table IV).

Conclusions

ELISA methods facilitate the identification of low contents of animal species (pork, beef, mutton and poultry) in meat products, even when sterilized. These methods are cheap, robust and easy to perform.

Turkey and chicken ELISA kits, which are recommended for raw products, also work for pasteurized products. Detection of chicken and turkey in sterilized products could be performed by DNA techniques, as specific probes are commercially available and limits of detection are satisfying (Bergwerff, 1999).

Limits of detection depend on many parameters, such as the origin of muscles, percentage of fat, maturity of meat, processing, ... They might be quite different for any other product. In practice, these methods are not quantitative.

References

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| Pork | Mutton | Absorbance at 405 nm | | |
|---------|---------|----------------------|-------------|------------|
| | | Raw | Pasteurized | Sterilized |
| 50.00 % | 0.00 % | 0.816 | 0.811 | 0.790 |
| 49.95 % | 0.05 % | 0.790 | 0.730 | 0.771 |
| 49.50 % | 0.50 % | 0.755 | 0.791 | 0.773 |
| 45.00 % | 5.00 % | 0.731 | 0.741 | 0.724 |
| 5.00 % | 45.00 % | 0.552 | 0.440 | 0.387 |
| 0.50 % | 49.50 % | 0.184 | 0.114 | 0.097 |
| 0.05 % | 49.95 % | 0.057 | 0.051 | 0.039 |
| 0.00 % | 50.00 % | 0.035 | 0.030 | 0.029 |

| Controls | Absorbance |
|----------|------------|
| pork | 0.595 |
| beef | 0.029 |
| mutton | 0.025 |
| poultry | 0.027 |

Table I. Immunodetection of pork in products containing pork and/or mutton. The threshold was set at 0.067. Means of duplicates are reported in bold type. Positive results are written on grey background.

| Beef | Turkey | Absorbance at 405 nm | | |
|---------|---------|----------------------|-------------|------------|
| | | Raw | Pasteurized | Sterilized |
| 50.00 % | 0.00 % | 0.768 | 0.719 | 0.520 |
| 49.95 % | 0.05 % | 0.723 | 0.670 | 0.497 |
| 49.50 % | 0.50 % | 0.738 | 0.673 | 0.540 |
| 45.00 % | 5.00 % | 0.710 | 0.643 | 0.496 |
| 5.00 % | 45.00 % | 0.648 | 0.514 | 0.273 |
| 0.50 % | 49.50 % | 0.424 | 0.210 | 0.084 |
| 0.05 % | 49.95 % | 0.156 | 0.080 | 0.056 |
| 0.00 % | 50.00 % | 0.049 | 0.053 | 0.058 |

| Controls | Absorbance |
|----------|------------|
| pork | 0.048 |
| beef | 0.561 |
| mutton | 0.118 |
| poultry | 0.043 |

Table II. Immunodetection of beef in products containing beef and/or turkey. The threshold was calculated as in table I: 0.174. Results are presented as in table I.

| Chicken | Turkey | Absorbance at 405 nm | | |
|---------|---------|----------------------|-------------|------------|
| | | Raw | Pasteurized | Sterilized |
| 50.00 % | 0.00 % | 0.129 | 0.047 | 0.048 |
| 49.95 % | 0.05 % | 0.170 | 0.051 | 0.049 |
| 49.50 % | 0.50 % | 0.436 | 0.141 | 0.047 |
| 45.00 % | 5.00 % | 0.732 | 0.611 | 0.047 |
| 5.00 % | 45.00 % | 0.798 | 0.649 | 0.038 |
| 0.50 % | 49.50 % | 0.862 | 0.696 | 0.045 |
| 0.05 % | 49.95 % | 0.826 | 0.652 | 0.049 |
| 0.00 % | 50.00 % | 0.810 | 0.722 | 0.045 |

| Controls | Absorbance |
|----------|------------|
| chicken | 0.040 |
| turkey | 0.376 |
| pork | 0.041 |

Table III. Immunodetection of turkey in products containing chicken and/or turkey. The threshold of detection was set at 0.200. Results are presented as in table I.

| Formulations (in %) | | | | | | Identification of animal species | | |
|---------------------|-------|-------|-------|-------|-------|----------------------------------|--------------------|------------|
| Pork | Mut. | Chi. | Tur. | Pou. | Beef | raw | pasteurized | sterilized |
| | | | 0.00 | 0.00 | 50.00 | Beef | Beef | Beef |
| | | | 0.05 | 0.05 | 49.95 | Beef Pou. | Beef | Beef |
| | | | 0.50 | 0.50 | 49.50 | Beef Pou. (tur.) | Beef Pou. | Beef |
| | | | 5.00 | 5.00 | 45.00 | Beef Pou. (tur.) | Beef Pou. (tur.) | Beef Pou. |
| | | | 45.00 | 45.00 | 5.00 | Beef Pou. (tur.) | Beef Pou. (tur.) | Beef Pou. |
| | | | 49.50 | 49.50 | 0.50 | Beef Pou. (tur.) | Beef Pou. (tur.) | Pou. |
| | | | 49.95 | 49.95 | 0.05 | Pou. (tur.) | Pou. (tur.) | Pou. |
| | | | 50.00 | 50.00 | 0.00 | Pou. (tur.) | Pou. (tur.) | Pou. |
| 50.00 | 0.00 | | | | | Pork | Pork | Pork |
| 49.95 | 0.05 | | | | | Pork | Pork | Pork |
| 49.50 | 0.50 | | | | | Pork Mut. | Pork | Pork |
| 45.00 | 5.00 | | | | | Pork Mut. | Pork Mut. | Pork Mut. |
| 5.00 | 45.00 | | | | | Pork Mut. | Pork Mut. | Pork Mut. |
| 0.50 | 49.50 | | | | | Pork Mut. | Pork Mut. | Pork Mut. |
| 0.05 | 49.95 | | | | | Mut. | Mut. | Mut. |
| 0.00 | 50.00 | | | | | Mut. | Mut. | Mut. |
| | | 50.00 | 0.00 | 50.00 | | Pou. (chi.) | Pou. (chi.) | Pou. |
| | | 49.95 | 0.05 | 50.00 | | ND (chi.) | ND (chi.) | ND |
| | | 49.50 | 0.50 | 50.00 | | ND (chi.) (tur.) | ND (chi.) | ND |
| | | 45.00 | 5.00 | 50.00 | | Pou. (chi.) (tur.) | Pou. (chi.) (tur.) | Pou. |
| | | 5.00 | 45.00 | 50.00 | | Pou. (chi.) (tur.) | Pou. (chi.) (tur.) | Pou. |
| | | 0.50 | 49.50 | 50.00 | | ND (chi.) (tur.) | ND (tur.) | ND |
| | | 0.05 | 49.95 | 50.00 | | ND (tur.) | ND (tur.) | ND |
| | | 0.00 | 50.00 | 50.00 | | Pou. (tur.) | Pou. (tur.) | Pou. |

Table IV. Identification of animal species in experimental meat products (summary). Mut.: mutton; Chi.: chicken; Pou.: poultry; ND: not done.