# DETECTION OF HEAD MEAT AND MECHANICALLY RECOVERED MEAT BY CHEMOMETRIC ANALYSIS OF PROTEIN ELECTROPHORETIC PROFILES – A FEASIBILITY STUDY

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**Background**

The use of head meat and mechanically recovered meat in processed meat products is motivated by financial gain in the case of MRM and by technological properties in the case of head meat. Detection of MRM is an important issue in terms of meat authenticity (1). Several methods are available to enable its detection, however they all rely on experienced evaluation and careful comparison with known standards (2). Chemometric analysis of electrophoretic protein profiles has not been used extensively, but we have previously shown that this approach can be used to identify and quantify animal species in mixtures of ground meat (3).

**Objectives**

This work aims at providing an analytical method for head meat and MRM which is reliable and does not depend on extensive operator experience, and which easily can be transferred between laboratories. Any success in this adds to the general applicability of chemometric analysis of electrophoretic patterns.

**Methods**

Beef meat was used throughout this study. Minced meat mixtures were made from (i) production meat, (ii) head meat and (iii) two qualities of MRM. Samples contained from 0 to 100% of each component, according to a simplex design illustrated in Fig.1. A total of 75 samples were made based on 12 entirely independent raw material samples. All samples were extracted with distilled water, centrifuged and applied to isoelectric focusing on a plate containing an immobilised pH-gradient (Immobiline 4-7 from Pharmacia). Gels were photographed by 35mm Ektachrome film and scanned by Kodak Norge AS onto a CD disk in ".pcd"-format. Image analysis procedures were performed with ImagePro v.1.3 (Media Cybernetics). For background correction, a background image was constructed by median filtering, and this image was subtracted from the original image. Further signal optimisation was obtained by horizontal edge filtering technique. Chemometric analyses were performed with The Unscrambler v.5.5. (CAMO AS). The method chosen was Principal Components Regression (PCR), validated by leverage correction. Data from gel protein profiles were X data and the composition of the samples were the Y data. For investigating effects of image processing manipulations, samples were modelled individually. For estimating the overall modellability of the system, the following steps were performed. Corresponding to each sample in the design there were 3 independent replicate samples. For each sample, a "super-sample" was constructed, at each position along the electropherogram taking the highest numerical value among the three independent profiles. Thus, the resulting profiles compensated to some extent for variation in protein composition between samples as well as for errors due to limited reproducibility of the isoelectric focusing. However, this set of data gives a balanced representation of the experimental system and should return a realistic multivariate model of the system.

**Results and discussion**

Spatial filtering of gel images by background correction and edge detection methods virtually eliminated background signals and enhanced signal strength from weak bands (Fig 2).

It was investigated to what extent the enhanced signal strength (Fig.2) improved the analytical result. Models based on background-corrected images was inferior to those based on background-corrected and edge-filtered images, in terms of explained Y-variance, correlation between measured and calculated Y-values as well as prediction error (Table 1). Combining two different types of MRM into one Y-parameter did not affect the quality of analysis to a great extent (not shown). This indicates that multivariate analysis of isoelectric focusing patterns may be successful in analysing MRM from several sources, which are known to have large differences in their composition (4).

The modelling strategy of constructing "super-samples" improved the analysis substantially (Table 2). This strategy explained 75 - 80% of the samples' compositional data. It had a correlation of 0,94 - 0,96 between measured and calculated values of MRM content, and the prediction error was in the order of 10%. The other analyte addressed in this study, head meat, could be predicted with a precision comparable to MRM. It could be observed from the isoelectric focusing gels that small variations in mobility occurred between samples and between gels. The fact that the "super-sample" strategy was the best one in this study indicates that correcting for this variation is a major key to better precision in this analysis. This strategy gave prediction with good linearity over the whole range of concentrations both for MRM (Fig 3) and head meat (Fig 4).The present study complements previous work (3) indicating that animal species can be determined using the same approach. In both studies, corrections of variations in mobility have been shown to be the limiting factor for precise analytical results. It is desirable to validate the present findings on a set of new samples.

**Conclusions**

Chemometric analysis of isoelectric focusing protein profiles has been shown applicable for the detection of MRM and head meat in minced meat mixtures. The limiting factor is to make mathematical corrections for variability in mobility between samples and between gels. This makes the present approach a promising one for several analytical problems that in one form or another rely on protein composition

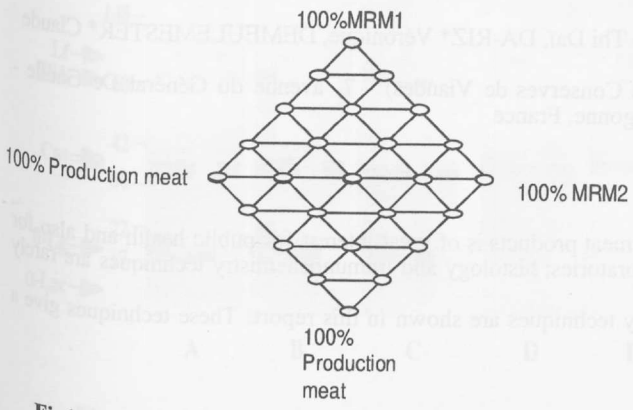


**Fig1.** Simplex design showing the composition of samples in the study.

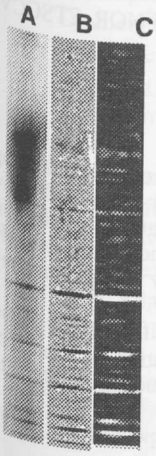


**Fig. 2**
Image processing of gel electropherograms. Lane A is the original image. Lane B has been background corrected. Lane C has additionally been edge filtered.

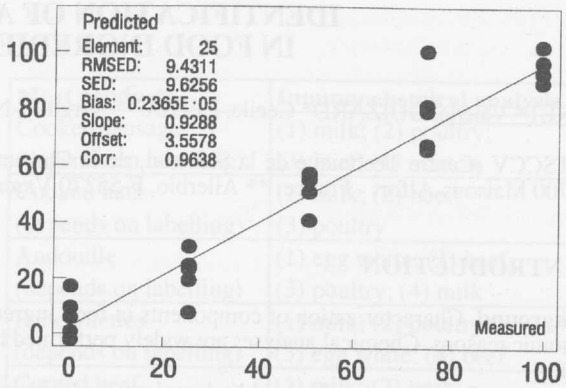


**Fig.3**
Prediction plot for mechanically recovered meat in the samples in the study

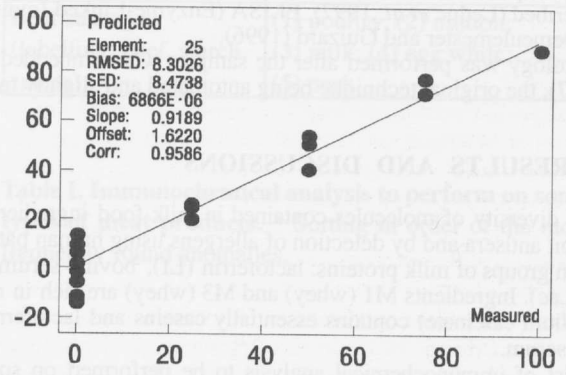


**Fig 4.**
Prediction plot for head meat in the samples in the study.

TABLE 1
*The effect of image processing on the multivariate model for predicting mechanically recovered meat*

| Filtering method | Explained Y-variance (% of total) | Optimum no of Principal Components | Correlation | Prediction error (%)** |
|---|---|---|---|---|
| Background filtered | 58 | 2 | 0.81 | 17.1 |
| Background and edge filtered | 64 | 8 | 0.90 | 12.7 |

** Prediction error is expressed as RMSEP (Root Mean Square Error of Prediction). Units are % reflecting the composition of the samples

TABLE 2
*The effect of modelling with "super-samples" on the multivariate model for predicting mechanically recovered meat*

| Data sets | Explained Y-variance (% of total) | Optimum no of Principal Components | Correlation | Prediction error (%)** |
|---|---|---|---|---|
| Individual samples | 75 | 7 | 0.94 | 10.0 |
| "Super-samples" | 82 | 7 | 0.96 | 9.4 |

** Prediction error is expressed as RMSEP (Root Mean Square Error of Prediction). Units are % reflecting the composition of the samples

**References**

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