SPECIES IDENTIFICATION OF MEAT MIXTURES BY ISOELECTRIC FOCUSING AND MULTIVARIATE DATA ANALYSIS

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Background

Methods for demonstrating that a meat product has not been adulterated are important for producers, authorities and consumers. The motivations of each might differ from that of the others, but the goal is common: the presentation and/or labelling of a meat product should reflect its nature and origin. One prime issue of authenticity is the one of species identification.

Electrophoretic methods, particularly isoelectric focusing, have been widely used for several decades for identifying meat species. (*E.g.* Zerifi, A., C. Labie, and G. Benard. (1991), Skare, K., B. Thorson, and T. Hoyem. (1969), Bauer, F. and A. Kelner. (1989)). However, they have been limited by the fact that electrophoretic profiles are complex, and virtually impossible to interpret for unknown mixtures. On this background we have combined isoelectric focusing with multivariate data analysis, in order to extract only the relevant information from the electrophoretic profiles.

Methods

Design of mixtures

This study used chicken (mechanically recovered), beef and pork meat. The design of mixtures were to span all possible combinations of the three species, so a "triangular" type of design was chosen, with intervals of 25%. The design is illustrated in Fig.1.

Preparation of meat mixtures and extraction of proteins

Meat from individual animals was ground mechanically and mixed by hand, according to the experimental design. From each mixture 2g was weighed out and suspended in 10ml glass distilled water. After 30min the samples were sentrifuged for 10min at 700g. The resulting supernatants were clarified by filtration through filters of pore size 0,8µm.

Isoelectric focusing (IEF)

Isoelectric focusing was performed in Immobiline 4 - 7 gels. The gel was rehydrated in glass distilled water supplemented with 2,5% Pharmalyte 4-6,5 for 2h. After mounting the gel on a Multiphor II apparatus, 20 µl of each protein extract was applied at the cathodic end of the gel. Electrophoresis was run at 3500V for 15000Vh. Subsequently, proteins were visualized by Coomassie R staining.

Digitalisation of electrophoretic profiles

The gel was scanned using a Princeton CCD camera. The resolution of the image was 512 x 512 pixels with a resolution of 16 bit. Along each lane of the gel a trace was recorded and converted to numerical values by use of ImagePro software. These records were used as the result of IEF being subject to multivariate analysis.

Multivariate data analysis

The IEF records were selected as the X-matrix. The composition values of the meat mixture design were selected as the Y-matrix. The X matrix was modelled together with the mixture Y-information. We have chosen PLS as the system for modelling.Because of the low number of objects we have modelled by full crossvalidation. This method validates each sample against all the other and the resulting cross-validated model represents the mean modelability of all the samples. As an error measure we have used the Root Mean Square Error of Prediction. (RMSEP) (Martens and Næs, 1989) which is closely related to the standard deviation.

Results and discussion

Fig. 2 shows a representative isoelectric focusing gel of all 15 samples. It appears that corresponding bands from different lanes have identical positions in the profile, as accurately as can be estimated visually. This would be expected to be a prerequisit for analysing the data digitally, so this type of electrophoresis seems to be qualified as a starting point for multivariate modeling

The result of multivariate analysis shows that pork is best described after 4 factors, chicken is best described after 3 factors while beef is best described after 5 factors. The precision by which the different predictions are done (RMSEP) is about 9 percent. Such a high presicion, even when a model is based on only 15 samples, implicates that this approach potentially is an acceptable analytical method for adulterated meat.

The relations between the objects (samples) are shown in the score plot of Fig.3. We observe that in this model the original mixture design is nearly reproduced in terms of the relative positions of the samples, particularly in the right half of the triangle. This is the region with high levels of chicken. There is a non linear area in the lower left corner. If further refinement of electrophoretic separation does not solve this problem, this region should be modelled with non-linear methods like neural networks.

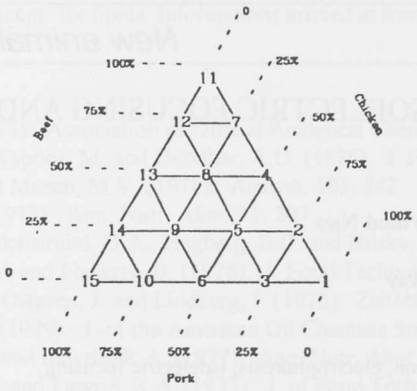

Figure 1.The experimental design showing the composition of all 15 samples in the study.

Figure 2. Coomassie-stained IEF gel of samples 1 - 15, applied from left to right.

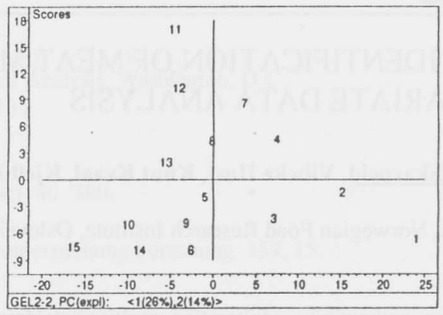

Figure 3. The score plot shows the relations between the objects (samples). Their relative positions in the original triangular shape mixture design is nearly reconstructed.

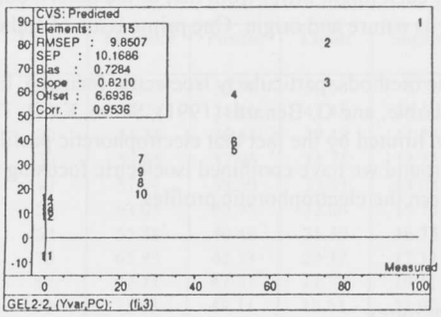

Figure 4. Predictons of chicken meat in mixtures of chicken. beef and pork meat.

Prediction of the different levels of chicken meat in all mixures analysed in this work, based on the model developed, is shown in Fig. 4. The response is nearly linear over the whole range of concentrations, although the lowest concentrations are not predicted very well. At present it is not clear whether this is due to fundamental limitations of the method, or to the low noumber of samples on which the model is based. Similar prediction plots were obtained for the other two species in the study - beef and pork.

Conclusions

Isoelectric focusing (IEF) of meat proteins combined with partial least squares (PLS) modelling is a powerful method to identify the species of meats in mixtures. This approach seems to overcome problems associated with visual inspection of IEF gel scans of complex mixtures of several species. It is, however, possible to enhance the modelling by using non-linear techniques.

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