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Detection of Adulteration in Cooked Meat Products by Mid-Infrared Spectroscopy

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Mid-infrared spectroscopy was used to discriminate between pure beef and beef containing 20% w/w of a range of potential adulterants (heart, tripe, kidney, and liver). Spectra were acquired from raw samples and from samples cooked using two different cooking regimes. Chemometric methods (principal component analysis, partial least squares regression, and linear discriminant analysis) applied to the spectra showed that discrimination between the pure and adulterated sample types was possible, irrespective of cooking regime. The cross-validated classification success rate obtained was ~97%. Discrimination between all five sample types (pure beef and beef containing one of each of the four adulterants) at each level of cook was also possible, but became more difficult as the cooking level increased.

KEYWORDS: Infrared; spectroscopy; beef; adulteration; cooked; offal; discrimination

INTRODUCTION

The nature of some meat products (pies, sausages, burgers) offers many possibilities for adulteration. Cheaper cuts or offal may be substituted for more expensive cuts, and water or vegetable matter may be added. In previous work (*1*), we conducted a feasibility study with the aim of determining whether mid-infrared (MIR) spectroscopy could be used for species identification, and if the method had potential as a rapid, low-cost, easy-to-use screening method for the food industry. We showed that it was possible to discriminate between three meats (turkey, chicken, and pork) and were additionally able to identify fresh samples from those that had been previously frozen. Subsequently, McElhinney et al. (*2*) have performed similar studies using red meats and a combination of analytical techniques.

Spectroscopy is not the only approach to such analysis. Other methods such as immunological (*3*, *4*), and enzymic methods (*5*) can be used, and may be both cheap and quick. However, the detection of nonmeat additives requires complex and more prolonged examination of the sample. The addition of offal can be particularly difficult to detect and may require detailed microscopic examination (*6*). We have recently shown that MIR spectroscopy, used in conjunction with appropriate chemometric methods, can discriminate between pure beef and beef mixed with selected offal (*7*). Furthermore, such methods could provide quantitative information if the potential adulterant was known.

A primary aim of the present work was to ascertain whether the discrimination obtained for raw meats could be obtained with cooked samples. We have again chosen to concentrate on the addition of offal to beef. However, we have elected to use only one type of cut (silverside), because the earlier study (*7*) showed that it was not readily possible to discriminate between different cuts of beef. We have also chosen to work at one adulterant level (20% w/w). This amount was chosen as being reasonably challenging, and representative of a realistic adulteration level. The detection limits for kidney and liver suggested by earlier work were around 10% w/w. In the present study, we have additionally included two other offal types, heart and tripe, the detection limits of which were not known in advance.

EXPERIMENTAL PROCEDURES

Samples. Samples of beef (cut: silverside), ox kidney, ox liver, ox heart, and tripe (stomach) were purchased from local retailers and butchers. The samples were minced to a coarse paste soon after purchase, using a Krups (Peoria, IL) coffee grinder, which was carefully washed between each preparation, using 0.2% Triton-X 100 solution followed by distilled water. Mixtures of offal and beef were prepared by adding 20% w/w of selected offal samples to randomly selected beef samples. Twelve samples of pure beef and of beef adulterated with each offal type, were used in the experiments. All samples were frozen at −20 °C after preparation, and thawed in the refrigerator before spectral acquisition or cooking.

Cooking. Samples were shaped into small patties of approximately 4 cm diameter and 1 cm thickness. These were cooked in a R-202M 800W IEC 705 microwave oven (Sharp Electronics Ltd, Manchester, UK) for 8 min, using either power setting 'medium-low' (240 W) or 'medium' (400 W). We will call these cooking regimes 'level 1' and 'level 2', respectively. The 'level 1' regime was just sufficient to cook the samples thoroughly, leaving no visibly raw areas. The 'level 2' regime cooked the samples thoroughly, without causing burning, but

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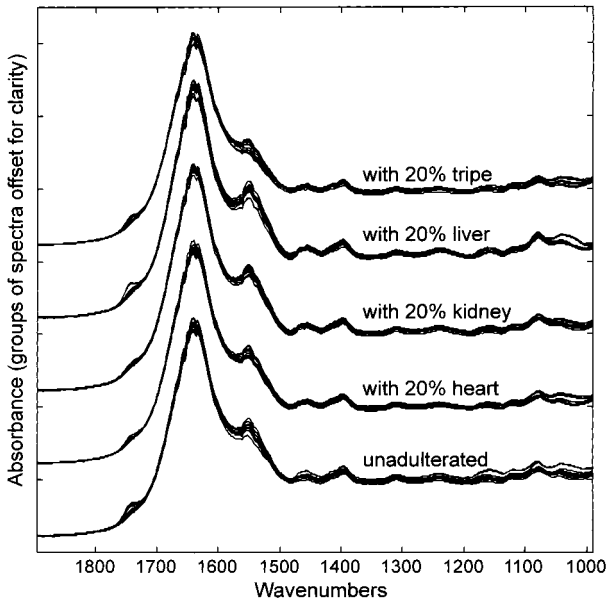

Figure 1. Infrared spectra of all uncooked samples.

with some dehydration. The cooked samples appeared to be of homogeneous color and texture throughout.

Instrumentation and Spectral Acquisition. All spectra were collected on a Spectra-Tech Applied Systems Inc (Shelton, CT) Monitir Fourier transform infrared (FTIR) spectrometer system equipped with a sealed and desiccated interferometer and room-temperature deuterated triglycine sulfate detector. An attenuated total reflectance (ATR) accessory was built into one of two dedicated sampling stations. The ATR crystal was removable from the instrument which remained sealed by the presence of potassium bromide windows. In this way, the crystal could be cleaned without ingress of water vapor into the spectrometer. The ATR crystal was a nominal 11-reflection zinc selenide crystal mounted in a trough plate. The crystal geometry was 45° parallelogram with mirrored angled faces.

Uncooked samples were applied to the ATR plate, taking care to achieve good contact between the sample and the ATR crystal, with no trapped air. A single-beam MIR spectrum of the sample was collected over the range 800 to 4000 cm^{-1} at a resolution of 4 cm^{-1}. Sixty-four interferograms were co-added before Fourier transformation using triangular apodization. The single-beam spectrum of each sample was converted to absorbance units using a single-beam background spectrum collected of a clean, dry ATR crystal.

Cooked samples were ground using the Krups coffee grinder after cooking and cooling to room temperature. The finely powdered, semi-dry material was spread onto the ATR crystal and held in place with gentle hand pressure applied to a rubber top-plate. Spectral acquisition conditions were the same as for the uncooked samples.

Between spectral acquisition of all samples, the ATR plate was cleaned with 0.2% Triton-X 100 solution, rinsed with distilled water, and then dried.

Chemometric Analysis. All data analysis was carried out using Matlab (The Mathworks Inc, Cambridge, UK). All absorbance spectra were truncated to 470 data points in the region 990−1895 cm^{-1}. Two chemometric methods were employed in this work. For data exploration, we have used principal component analysis (PCA) using the covariance matrix. For modeling the group structures in the data, we have used a combination of methods: partial least squares (PLS) regression onto dummy variables representing the proposed group structure

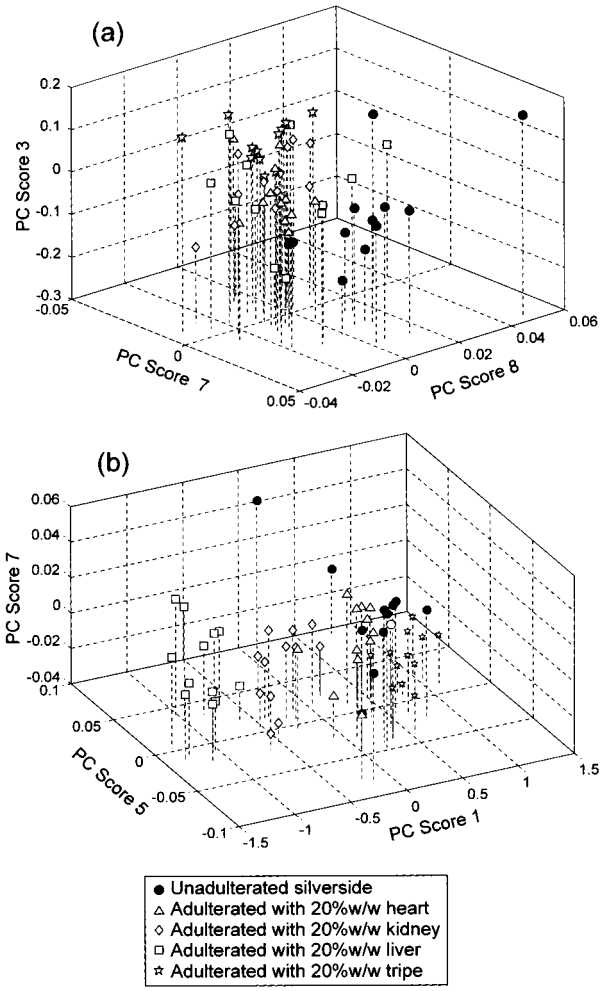

Figure 2. PCA of 'uncooked' data set: (a) the third vs seventh vs eighth PC scores, and (b) the first vs fifth vs seventh PC scores.

("discriminant" PLS (*8*)), followed by distance-based linear discriminant analysis (LDA), using the Mahalanobis distance metric, applied to subsets of the PLS scores. PLS-LDA model performance was measured by classification success rates obtained by "leave-one-out" or internal cross-validation (ICV), which has been shown to give a realistic indication of model performance (*9*).

RESULTS AND DISCUSSION

Spectra collected from all uncooked samples are shown in **Figure 1**. The spectra of raw beef, beef with kidney, and beef with liver were similar in appearance to those collected in earlier work (*7*). It is not possible to distinguish the spectra of adulterated from unadulterated samples by visual inspection alone.

A first step was to perform an exploratory principal component analysis (PCA), to examine the data from a different perspective. The PCA was performed using the covariance matrix. Plots of the first twenty PC scores against one another were examined. Some clustering of the data according to sample type was found in several of the PC dimensions. In general, at least two dimensions were needed to reveal a distinct group corresponding to a particular sample type. For example, in **Figure 2a**, the third, seventh, and eighth PC scores are plotted against one another. This plot is best able to separate the pure beef from the adulterated samples. **Figure 2b** shows the first,

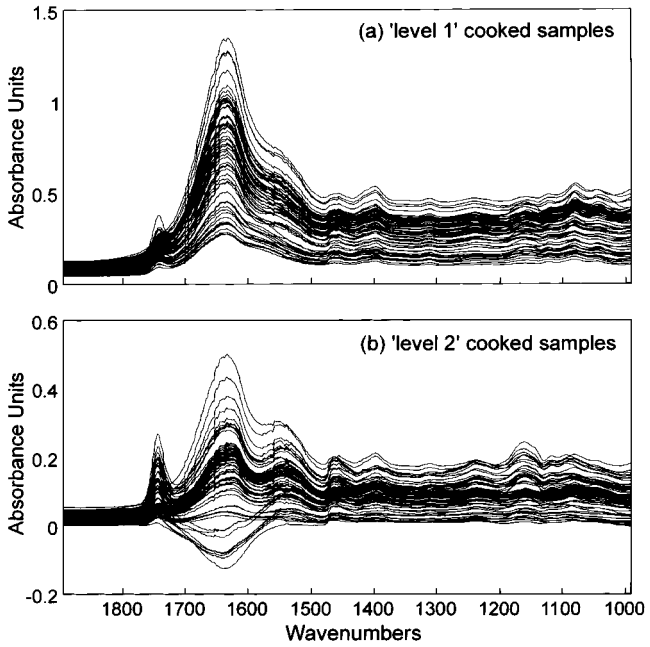

Figure 3. Infrared spectra of (a) 'level 1' and (b) 'level 2' cooked samples.

fifth, and seventh PC scores, which perhaps best simultaneously distinguish all five groups. It is interesting to note that in **Figure 2b**, the groups corresponding to the two new adulterant types used in this study, heart and tripe, lie closest to the 'unadulterated' group.

The spectra obtained from the cooked samples are shown in **Figure 3**. They are visually quite different from those obtained from raw meats. Some of these differences reflect compositional changes that occur upon cooking. In terms of spectral information, the most notable effect of cooking is dehydration, leading to the loss of the water peak at 1650 cm^{-1}, and relatively more prominent fat (1725 cm^{-1}) and protein (1650 and 1550 cm^{-1}) peaks. It proved to be more difficult to acquire good quality spectra from the drier cooked materials. The increased variability in the spectral baseline, and in the overall spectral intensity, are due to the difficulty of obtaining reproducible contact and coverage of the ATR crystal. This variability was found to be especially pronounced for the pure beef samples.

PCA exploratory analysis was applied to the 'level 1' and 'level 2' data sets. It was again possible to identify PC dimensions that separated the different sample types into the distinct groups. It is interesting to note that the distinction between the 'unadulterated' and 'adulterated' groups appeared greatest for the 'level 2' cooked samples, for which the first and second PCs alone are almost able to distinguish the pure beef samples (**Figure 4a**). Examination of the first loading (**Figure 4b**) shows that this distinction is based largely on differences in water content, consistent with the observed difference in response to cooking between unadulterated and adulterated samples.

PLS-LDA modeling was used to determine whether the discrimination between pure beef and offals obtained in our earlier work (*7*) could also be achieved for the groups of samples in the present study, which contain only 20% w/w of each offal type. 20% w/w is above the detection limit of 10% w/w estimated for liver and kidney adulteration in our previous work, but represents a sufficiently challenging and realistic level of adulteration, particularly with regard to the additional adulterants introduced in this study.

Initially, PLS-LDA was performed separately on the 'uncooked', 'level 1' and 'level 2' data sets. In each case, models

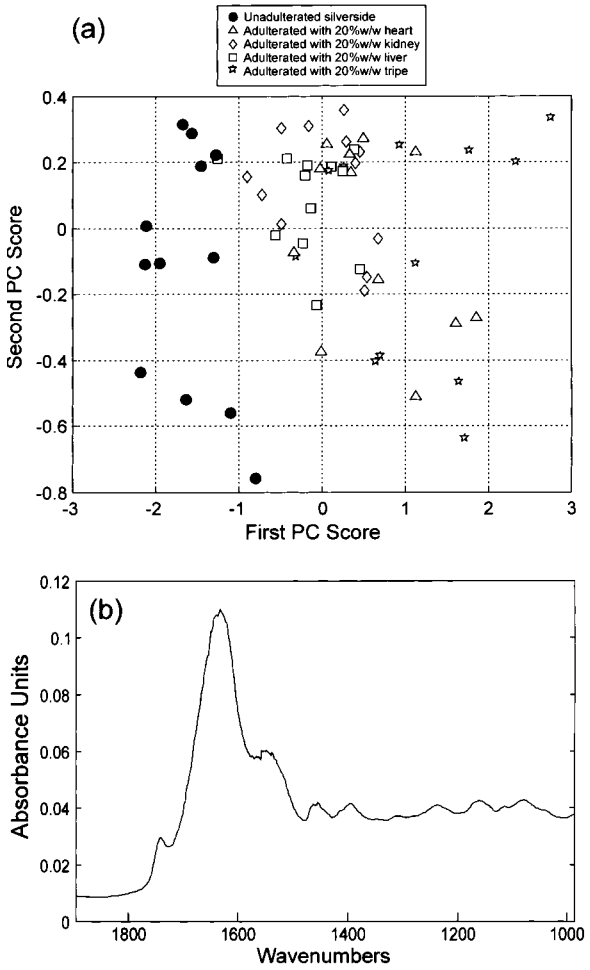

Figure 4. PCA of 'level 2' data set: (a) first vs second PC scores, and (b) first PC loading.

were obtained using subsets containing from 1 to 20 PLS scores. The ICV classification success rates are plotted versus model dimensionality in **Figure 5a**. For the 'uncooked' and 'level 1' data sets, the classification success rate reaches a maximum when 13 PLS scores are used when 59/60 and 57/60 correct classifications are obtained, respectively. For the 'level 2' data set, 14 PLS scores are required to give a success rate of 55/60. From visual consideration of **Figure 5a** as a whole, it appears that distinguishing all five groups becomes more difficult as the degree of cooking increases. The relatively high subset sizes needed to achieve good classification success rates reflect the complexity of the problem, and are broadly consistent with our findings from earlier work where, for example, 8 PLS scores were needed to optimize the calibration for added kidney in beef.

In practice, a more pragmatic aim is simply to distinguish adulterated from unadulterated samples. This reduces the analyses to two-group problems, viz, 'unadulterated' and 'adulterated'. PLS-LDA was applied to each of the 'uncooked', 'level 1' and 'level 2' data sets, this time as two-group formulations. The success rates obtained, again by ICV, are shown in **Figure 5b**. For all three cooking levels, it was possible to discriminate between the 'unadulterated' and 'adulterated' groups, with useful classification success rates. It also appears that distinguishing the 'unadulterated' and 'adulterated' groups is easiest in the 'level 2' data set, for which a maximum success rate of 59/60 was obtained from just 5 PLS scores. This is to be expected, in view of the results of the exploratory PCA analysis, and the observed difference in the morphology of pure

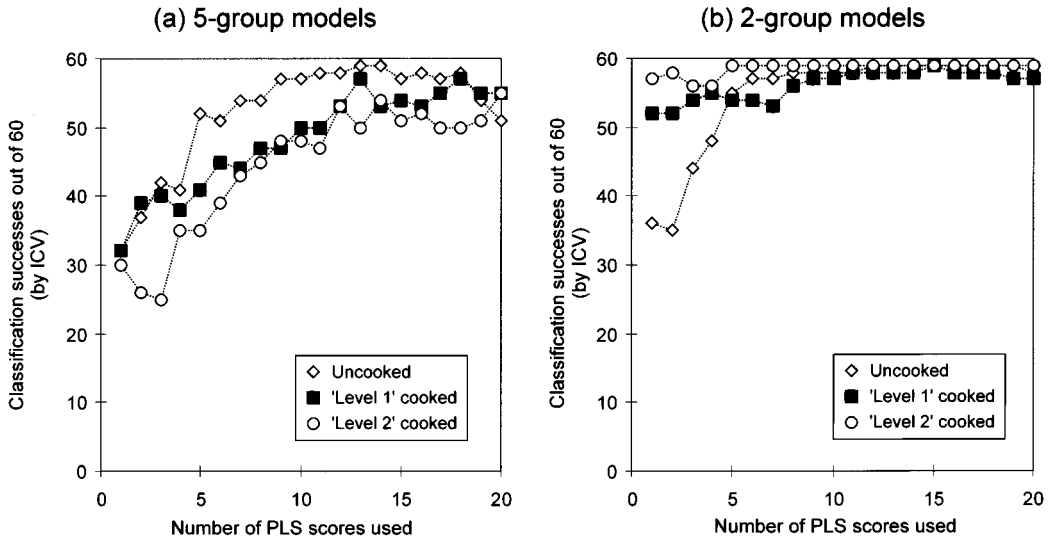

Figure 5. Classification success rates by ICV plotted versus model dimensionality, for (a) five-group discrimination, and (b) two-group discrimination.

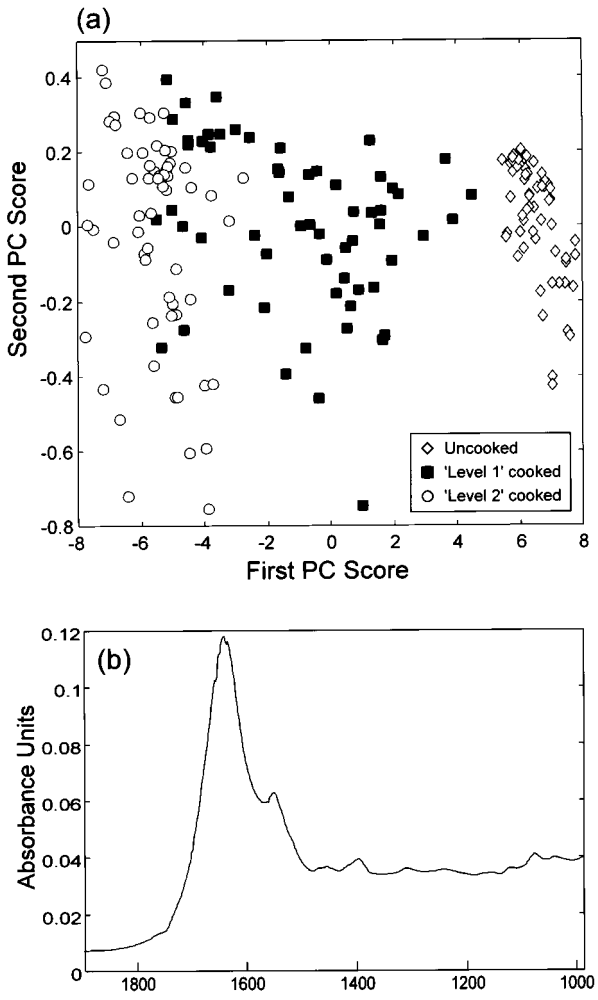

Figure 6. PCA of complete set of 180 spectra: (a) first vs second PC scores, and (b) first PC loading.

and adulterated cooked samples. The 'uncooked' and 'level 1' data sets required 12 and 15 scores, respectively, to achieve the same classification performance. We surmise that in these cases, the distinction between the two groups is based upon more subtle compositional changes, although it is beyond the scope of the present work to determine the exact nature of these differences.

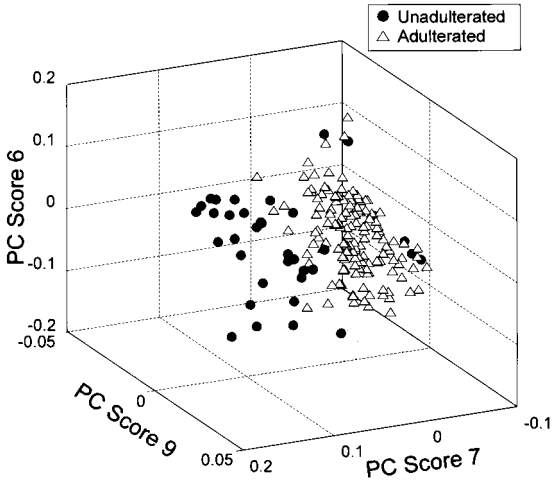

Figure 7. Sixth vs seventh vs ninth PC scores from the entire data set.

It is of interest to determine whether such discrimination between unadulterated and adulterated samples can be achieved from the complete data set. Such a model would be needed in a working adulteration screening test, where the amount of heating received by a product may not be known. PCA was applied to the complete set of 180 spectra. The first PC accounts for in excess of 99% of the variability in the data set. From visual examination of the scores with respect to this axis, it is clearly strongly associated with cooking level (**Figure 6a**). This scores plot also illustrates the increased variability at higher cooking levels. Examination of the first loading (**Figure 6b**) showed that this PC again represents mainly variation in the water content, consistent with the findings from visual inspection of the spectra. Note that it is also highly similar to the first PC loading from the 'level 2' data set (**Figure 4b**). Among the PC scores with lower variances, some were identified that were able to distinguish, at least partially, the 'adulterated' from 'unadulterated' groups (**Figure 7**).

PLS-LDA was applied, again using cross-validation for a two-group model. The classification success rate is shown versus model dimensionality in **Figure 8**. We see that a useful classification success rate of 174/180 is obtained from 15 PLS scores. A graphical impression of the performance of the model can be obtained by examining the ICV predictions of the dummy variable made by the PLS regression (*10*). These are plotted against sample number in **Figure 9**. The boundary between the

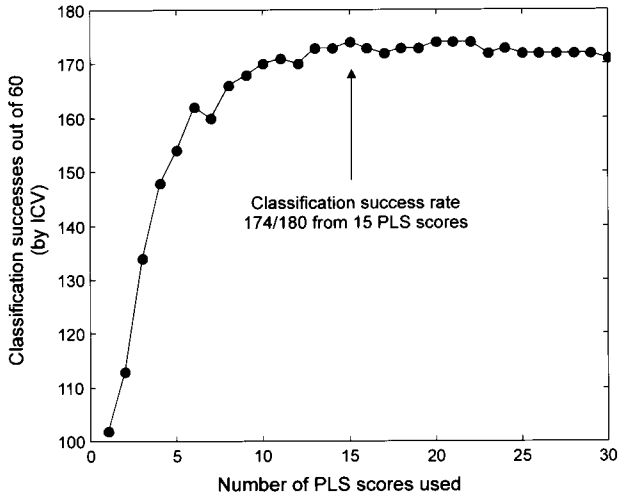

Figure 8. Classification success rate by ICV versus model dimensionality, for 2-group PLS-LDA (applied to complete data set).

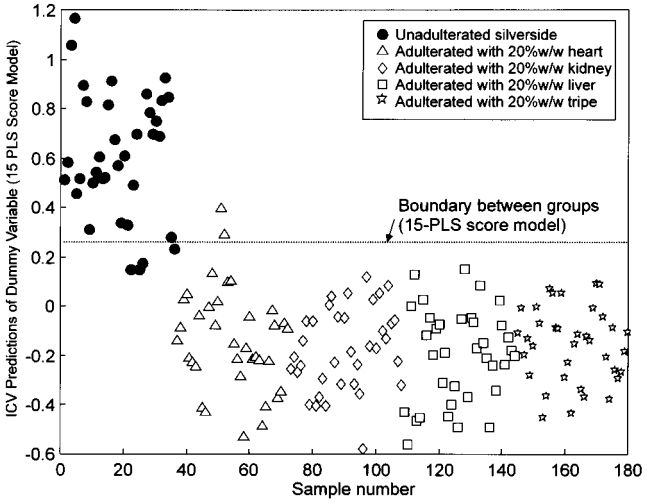

Figure 9. ICV predictions of the dummy variable obtained from PLS regression (15-score model).

two groups determined by LDA and a 15-score model is also indicated. Note that the two adulterated samples erroneously classified as unadulterated are both mixtures of beef with heart. This is consistent with the suggestion from the exploratory PCA that heart is closer in composition to beef than, say, kidney or liver.

CONCLUSIONS

This work has shown that it is possible to discriminate between pure beef and beef containing 20% w/w of a range of potential adulterants (heart, tripe, kidney, and liver), with acceptable classification success rates. This discrimination is possible both for raw samples and for samples cooked using two different regimes, although for the cooked samples it becomes more difficult to distinguish between the individual adulterants.

Cooking produces compositional changes in the samples, which lead to marked changes in the spectra obtained. The main compositional change related to cooking is water loss, and this is reflected in the spectra (reduction in the water peak at 1650 cm^{-1}, and relatively more prominent fat (1725 cm^{-1}) and protein (1650 and 1550 cm^{-1}) peaks), as well as in the results of the chemometric analysis. Good quality spectra are harder to obtain at the highest cooking level, and the spectra become more variable. This is due to experimental difficulties, in particular of ensuring reproducible contact with the ATR crystal, as the samples become drier and more particulate.

Internally cross-validated PCA/LDA analysis of the complete data set showed that 174/180 (~97%) of samples could be correctly classified as unadulterated or adulterated, irrespective of cooking level. We conclude that MIR spectroscopy combined with chemometrics has the potential to form the basis of a method for authenticity screening in cooked meat products.

LITERATURE CITED

(1) Al-Jowder, O.; Kemsley, E. K.; Wilson, R. H. Mid-infrared spectroscopy and authenticity problems in selected meats: a feasibility study. *Food Chem.* **1997**, *59*(2), 195−201.

(2) McElhinney, J.; Downey, G.; O'Connell, C. Quantitation of lamb content in mixtures with raw minced beef using visible, near-infrared and mid infrared spectroscopy. *J. Food Sci.* **1999**, 587−591.

(3) Jones, S. J.; Patterson, R. L. S. Modified indirect ELISA procedure for raw meat speciation using crude antispecies antisera and stabilized immunoreagents. *J. Sci. Food Agric.* **1986**, *37*, 767−775.

(4) Smith, C. J. Applications of immunoassay to the detection of food adulteration. An overview. In *Food Safety and Quality Assurance-Applications of Immunoassay Systems*; Morgan, M. R. A., Smith, C. J., Williams, P. A., Eds.; Elsevier Applied Science: London, 1991; pp 13−32.

(5) Sharma, N. K.; Srivatava, V. A.; Gill, J. P. S.; Joshi, D. V. Differentiation of meat from food animals by enzyme assay. *Food Control* **1994**, *5*, 219−221.

(6) Lumley, I. D. Authenticity of meat and meat products. In *Food Authentication*; Ashurst, P. R., Dennis, M. J., Eds.; Chapman and Hall: London, 1996; pp 108−139.

(7) Al-Jowder, O.; Defernez, M.; Kemsley, E. K.; Wilson, R. H. Mid-infrared spectroscopy and chemometrics for the authentication of meat products. *J. Agric. Food Chem.* **1999**, *47*, 3210−3218.

(8) Kemsley, E. K.; Discriminant Analysis of High-Dimensional Data: A Comparison of Principal Components and Partial Least Squares Data Reduction Methods. *Chemom. Intell. Lab. Syst.* **1996,** 33 *(1)*, 47−61.

(9) Defernez, M.; Kemsley, E. K. The use and misuse of chemometrics for treating classification problems. *Trac-Trends Anal. Chem.* **1997**, *16*, 2216−221.

(10) Kemsley, E. K.; Holland, J. K.; Defernez, M.; Wilson, R. H. Detection of Adulteration of Raspberry Purees using Infrared Spectroscopy and Chemometrics. *J. Agric. Food Chem.* **1996**, *44*, 3864−3870.

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