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RAPID COMMUNICATION

Mid-infrared spectroscopy and authenticity problems in selected meats: a feasibility study

O. Al-Jowder,[a] E. K. Kemsley[b] & R. H. Wilson[b]

[a]*University of Bahrain, College of Science, Chemistry Department, Isa Town, Bahrain*
[b]*Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, UK*

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This paper describes the results of a feasibility study into the use of mid-infrared spectroscopy for addressing certain authenticity problems with selected fresh meats. Preliminary analyses for meat speciation, the detection of 'frozen–thawed' meat, and semi-quantitative analysis of meat mixtures are reported. Fourier transform mid-infrared spectroscopy, attenuated total reflectance sample presentation, principal component analysis and partial least squares regression were used. It was possible to distinguish minced chicken, pork and turkey meats from their infrared spectra, and for each meat species it was possible to differentiate between fresh and frozen–thawed samples. Mid-infrared spectroscopy was also able to semi-quantitatively measure the levels of turkey and pork mixed with chicken meat. The method, which is rapid and easy to use, could with further development have the potential for authentication and quality control of meat products. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The determination of food authenticity and the detection of adulteration are major issues in the food industry, and are attracting an increasing amount of attention. With meat and meat products, major authenticity issues concern the substitution of high value raw materials with cheaper materials such as less costly cuts, mechanically recovered meat, offal, blood, water, eggs, gluten or other proteins of animal or vegetable origin. The latter can give rise to food safety concerns since such additions can cause allergic responses in certain individuals. There is also a problem with differentiating meat that has been frozen-and-thawed from fresh meat. The economic consequence of meat regulation abuse could be severe, but the exact level is difficult to assess. In the UK, the 1984 Meat and Meat Products legislation specifically prohibits adulteration of one type of meat with that from other species. In some countries the consumption of certain meats (e.g. pork) is proscribed for religious reasons. Therefore, analytical methods have focused on the identification of meat species in raw, cooked and processed products.

Meat speciation has been addressed by immunological (Jones & Patterson, 1986; Smith, 1991) and enzymic procedures (Sharma *et al.*, 1994). These methods along with electrophoretic techniques have also been used to differentiate fresh from 'frozen–thawed' meat which is another key authenticity issue (Siebert *et al.*, 1994). These methods have been considerably improved in terms of ease of use and length of analysis and can be used by untrained personnel. They are cheap and have the ability to detect a wide range and low levels of adulteration. However, one must often have an idea of the potential adulterant, and quantification of mixtures is a more complex issue.

Spectroscopic methods are an attractive option, fulfilling many analytical requirements such as speed and ease of use. Of these, mid-infrared methods (Wilson & Goodfellow, 1994) have recently been applied to the authentication of a range of materials, including fruit purees (Defernez *et al.*, 1995), jam (Defernez & Wilson, 1995), olive oil (Yoke Wah *et al.*, 1994) and coffee (Briandet *et al.*, 1996). In a number of cases, it has been shown that sample species can be determined, and multiple adulterants identified and quantified; in certain cases, adulterants not included during the development of the method can also be detected. Underlying the success of these applications is the fact that mid-infrared spectroscopy reports on a very large number of analytes simultaneously, and that the absorption bands are sensitive to the physical and chemical states of individual

constituents. Moreover, constituents present in quite low levels can be measurable and influence the discrimination ability of the technique (Yoke Wah *et al.*, 1994). Consequently, quite subtle compositional differences between samples can lead to good discrimination and identification. In the case of meat, the variation in protein and lipid structure and type might be expected to be apparent in the infrared absorption spectrum. However, these differences will not necessarily be evident on visual inspection, and multivariate statistical methods may be required to extract the useful information.

The aim of the work reported in this paper was to determine whether infrared spectroscopy is a potential candidate for meat authentication. We have carried out preliminary investigations related to the identification of selected meat species (chicken, turkey and pork), the ability to quantify these meats in mixtures, and the ability to detect frozen–thawed meat. Although there are certainly a number of methods available for speciation, it is very important to establish that spectroscopy can differentiate between species before attempting more complex problems. It is realised that the development of a fully validated method for detecting adulteration will require the generation of a very large database comprising samples of meat from different cuts, different organs and tissues, from more animals, and representing greater seasonal, regional and dietary variation. For this initial study chicken and turkey breasts were used, and all pork was lean meat taken from chops from the same region of the pig.

EXPERIMENTAL

Meat speciation and fresh/frozen–thawed studies

Twenty fresh chicken breast pieces and 20 fresh pork chops were purchased from four local food retailers (five pieces from each store). Twenty turkey breast pieces were purchased from a local butcher. The samples were bought at intervals over a period of 2 weeks. All samples (approximately 100 g) were minced on the day of purchase, using a Krups coffee blender, which was carefully washed and dried after processing each sample, using detergent solution (2% Triton-X) and distilled water. Mincing was carried out after removing the bones, skin and as much fat as possible. Only lean pork cuts were used. The total time of processing was approximately 5 min and the samples were monitored to ensure that they were not heated during this time. Each minced sample was divided into four approximately equal quantities: one of these was stored overnight at $< 10°C$ and infrared spectra were recorded the following day; the remaining three sub-samples were stored at $-30°C$. One of the three frozen quantities was defrosted in the refrigerator after approximately 15 days of storage, and infrared spectra were recorded the following day in this frozen–thawed condition.

All spectra were collected on a Spectra-Tech (Applied Systems Inc.) Monitir Fourier transform infrared (FTIR) spectrometer system, fitted with a sealed and desiccated interferometer and a room temperature deuterated triglycine sulphate (DTGS) detector. An overhead attenuated total reflectance (ATR) accessory was built into one of two dedicated sampling stations. The accessory comprised transfer optics within a desiccated chamber, sealed from the atmosphere by two potassium bromide windows. Through these windows the infrared radiation was directed into the detachable ATR element. The element used was a (nominal) 11-reflection zinc selenide crystal mounted into a plate with a shallow trough for sample containment. The potassium bromide windows allowed the ATR plate to be removed for cleaning without ingress of water vapour into the spectrometer. The crystal geometry was a 45° parallelogram with mirrored angle faces.

For spectral acquisition, the samples were spread directly onto the ATR element. Spectra were recorded from 800 to 4000 cm^{-1}. For each spectrum, 64 interferograms were co-added and a triangular apodization was employed before Fourier transformation. Each single-beam sample spectrum was ratioed to a single-beam spectrum of the clean ATR plate collected under identical conditions, and converted into absorbance units. The ATR plate was thoroughly cleaned between each sample by removing the previous sample with tissue, and cleaning with 2% Triton X-100 solution and distilled water. This procedure was found to efficiently remove traces of fat from the ATR crystal.

Both fresh and frozen–thawed samples were divided into two equal portions, and spectra recorded of each, giving a total of 40 spectra for each meat species for both fresh and frozen–thawed samples. The number of samples and spectra recorded are summarised in Table 1.

Table 1. Number and types of meat samples and spectra

Meat	Source identifier code					Number of pieces	Number of samples obtained		Spectra	
	A	B	C	D	E		Fresh	Thawed	Fresh	Thawed
Chicken	5	5	5	5	–	20	20	20	2×20=40	2×20=40
Pork	5	5	5	5	–	20	20	20	2×20=40	2×20=40
Turkey	–	–	–	–	20	20	20	20	2×20=40	2×20=40
									Total: 120	Total: 120

Quantification study

For the investigation of the ability of infrared spectroscopy to quantify meat mixtures, chicken was blended with pork or turkey, in the range 10–90% (w/w) chicken in increments of 2%. Frozen–thawed sub-samples remaining from the speciation study were used to prepare the mixtures. Different combinations were made of chicken, turkey and pork from the various retail sources used. The mixtures were prepared by carefully blending meats after mincing. In total, 41 mixtures of chicken with pork, and 41 mixtures of chicken with turkey, were prepared. Infrared spectra were collected of each mixture using the protocol as described.

Data analysis

For the data analysis, all spectra were truncated to 448 data points in the region 1000≃1800 cm^{-1}. Principal component analysis (PCA) was carried out using WinDiscrim software (E. K. Kemsley, Norwich). Partial least squares (PLS) regression was carried out using MatLab software (The MathWorks, Inc., Natick, MA) and a macro for orthogonal PLS regression using full internal cross-validation (Martens & Naes, 1989) written by the authors.

For quantitative analysis, the spectra of meat mixtures were regressed using PLS onto the pork and turkey concentration data. Again, spectra were pretreated with baseline correction and area-normalisation. Full 'leave-one-out' cross-validation was carried out: for each of the series, PLS regression was performed using 40 of the spectra, whilst the 41st was left out of the analysis to act as a 'test' sample; this procedure was repeated 41 times with a different spectrum left out each time. Thus, a prediction value was obtained for all samples and, hence, an impression of the performance of the regression. An important consideration when employing PLS is the number of 'factors' to use in the regression step: we refer to the text by Martens & Naes (1989) for a description of this and other issues concerning PLS, and simply state that the criterion used to select the optimum regression model was minimum cross-validation error. For both series, this was found to occur at six factors.

RESULTS AND DISCUSSION

The raw spectra of fresh minced chicken, pork and turkey meat are shown in Fig. 1. To the eye, the spectra of each species appear to be similar. The major feature at about 1650 cm^{-1} arises from water (O–H stretch) with a significant underlying contribution from protein (amide I). The second largest peak is the amide II absorption of protein. A small feature can be seen at about 1740 cm^{-1} due to fat (C=O ester). This peak is relatively more intense in the spectra of pork meat. For data processing the spectral region 1800–1000 cm^{-1} was chosen as this usually contains the most information and reduces the processing time for multivariate analyses.

Some variation of overall intensities was found within the spectra of the duplicate portions. This was probably due to variations in the efficiency of contact with, and coverage of, the ATR crystal. Therefore, we elected to use a data pretreatment which can be useful for mitigating this effect: baseline correction following by normalisation on the integrated spectral area. The results of a PCA using the pretreated spectra of the fresh meats are shown in Figs 2 and 3. In this case, scores on only the first two principal components (PC) are sufficient to visibly separate the spectra of each meat, although with only two dimensions there is some obvious overlap (Fig. 2). Examination of PC loadings can often be diagnostic for the source of such clustering. The first two loadings (shown in Fig. 3) can only be partially interpreted. It is clear that lipid and protein contents are important factors. Loading 1 shows characteristic protein absorptions at about 1650 and 1550 cm^{-1}. The PC scores associated with this loading are generally greater for chicken than turkey which are greater than pork. This order is consistent with the typical protein contents of these meats (Paul & Southgate, 1978). The second PC loading has features arising from lipid at about 1740 cm^{-1}, as well as less interpretable protein-associated absorptions, which may indicate that the amino acid composition of the protein, or indeed protein secondary structural differences may be important.

PCA was applied to the spectra (Fig. 4) of the frozen–thawed meats in the same way. Grouping of the data was again observed, but there appeared to be more overlap between the chicken and turkey samples (Fig. 5). This implies that the specific freeze–thaw process used in this work has affected the meat in a way that can be detected spectroscopically. The PC loadings associated with this discrimination (Fig. 6) are generally similar to those produced by fresh samples, although some extra features were seen in the region 1800–1500 cm^{-1}.

Further analyses were undertaken to see if it was possible to distinguish between fresh and frozen–thawed samples. Each meat species was treated individually, and three separate PCAs carried out. The results showed that it was possible to achieve good separation of fresh and frozen–thawed meats for all three meats studied. An example, chicken, is shown in Fig. 7. The clustering is striking: with the exception of a single sample, there is a clear division of the data into the fresh and frozen–thawed groups (it should be remembered that most of the samples were purchased in a supermarket and no absolute guarantees could be made about freshness). The PC loadings responsible for the differentiation were not interpretable, but we concluded that the freeze–thaw process had induced significant chemical differences between the samples. The features observed in the PC loadings suggested that differentiation may result from protein conformational changes induced by freezing but this is difficult to prove.

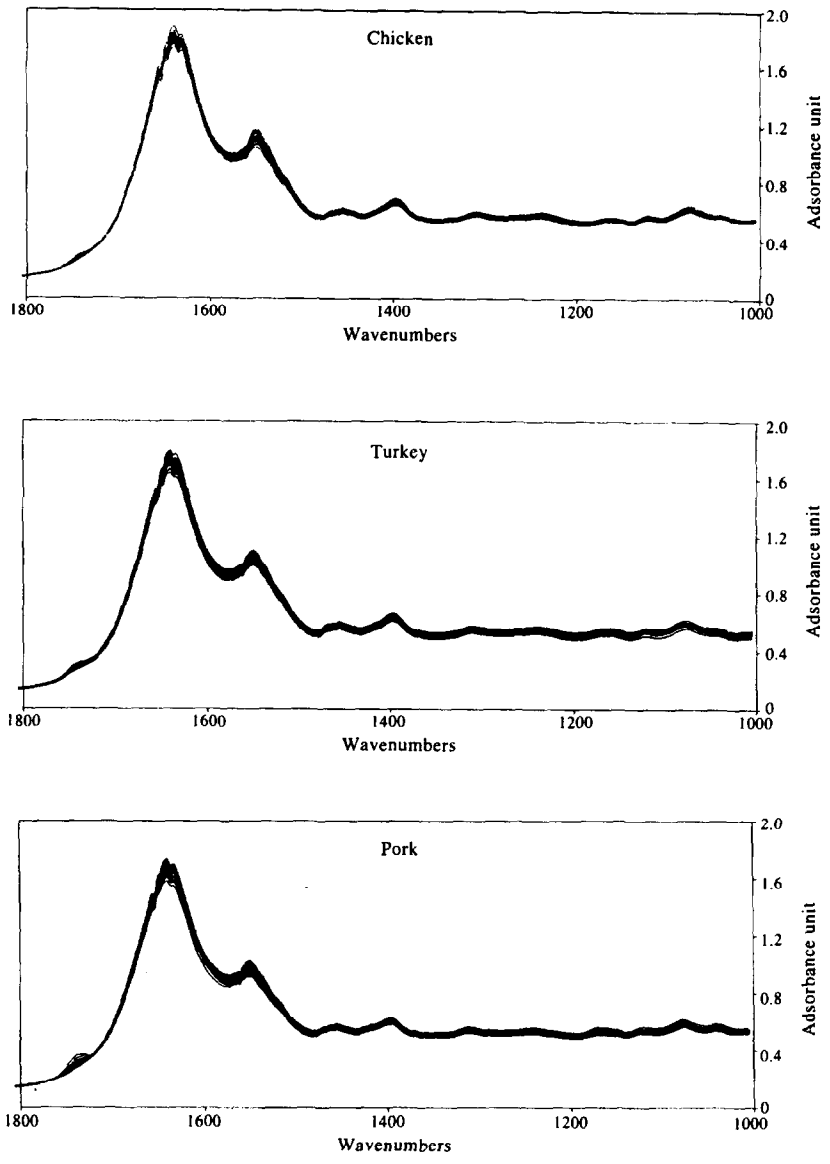

Fig. 1. Raw infrared spectra (1800–1000 cm^{-1}) of minced fresh chicken, turkey and pork meat.

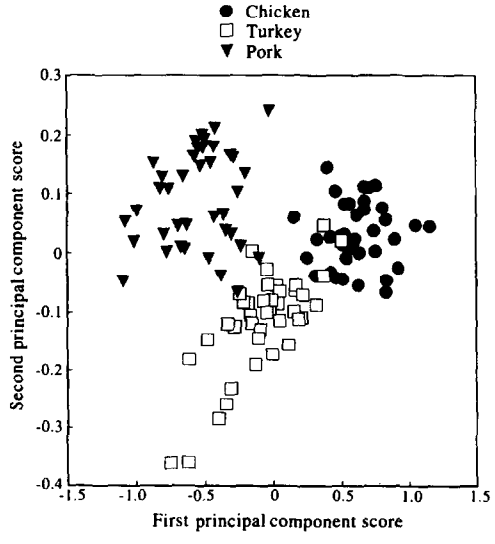

Fig. 2. Principal components scores plots (PC 1 versus PC 2) based on spectra shown in Fig. 1.

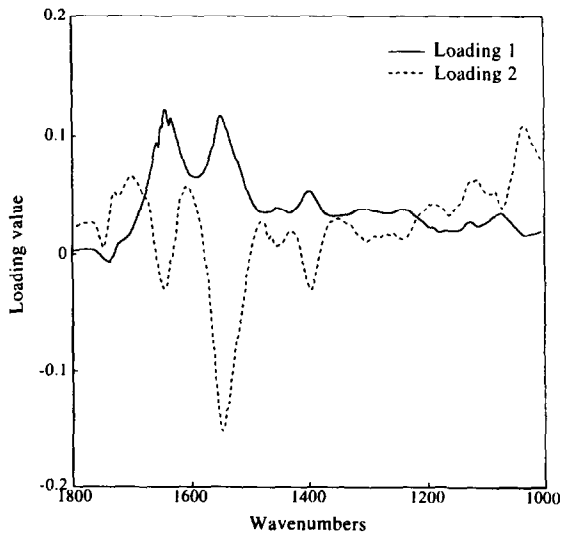

Fig. 3. First and second principal components loadings associated with PC scores used in Fig. 2.

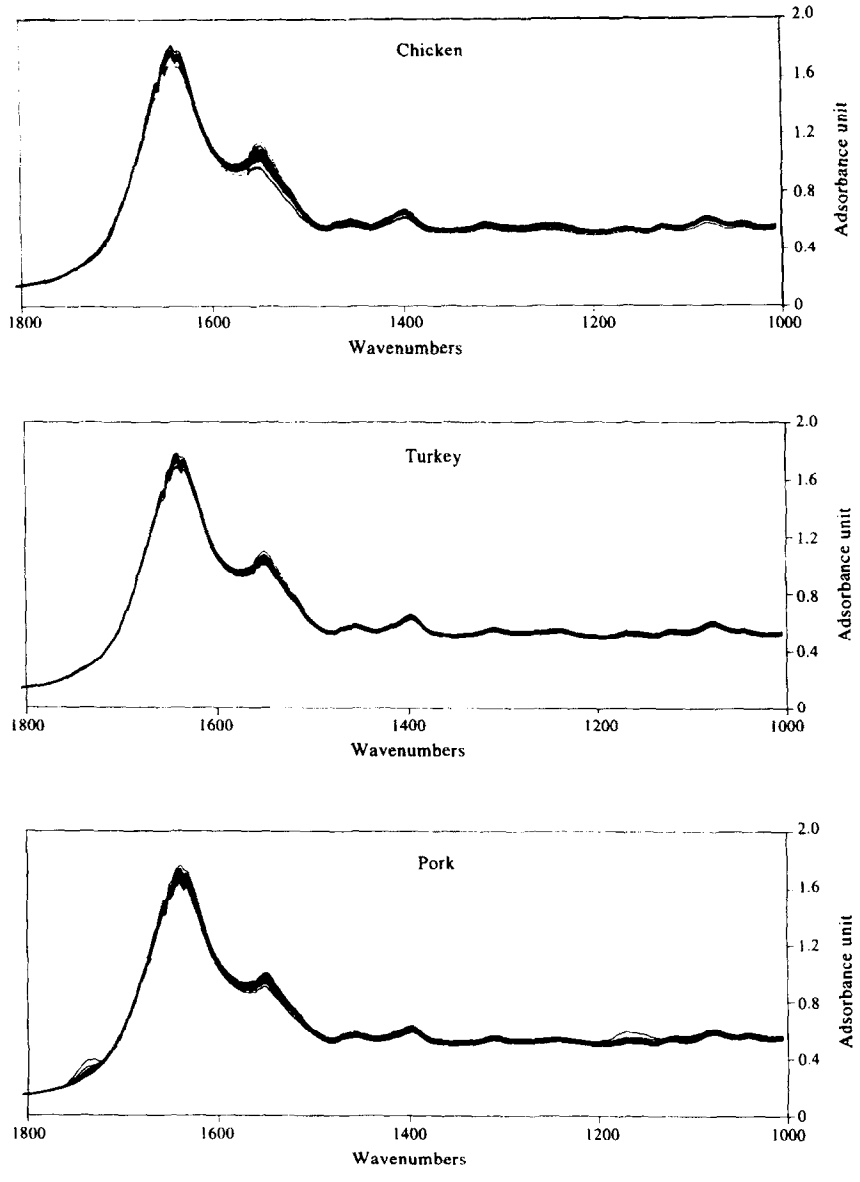

Fig. 4. Raw infrared spectra (1800–1000 cm^{-1}) of minced frozen–thawed chicken, turkey and pork meat.

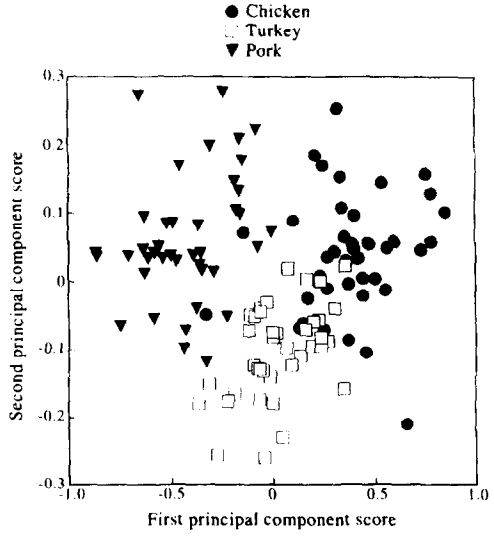

Fig. 5. Principal components scores plots (PC 1 versus PC 2) based on spectra shown in Fig. 4.

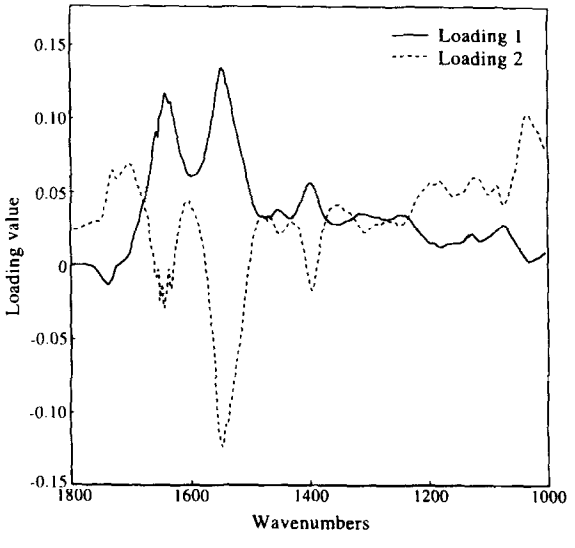

Fig. 6. First and second principal components loadings associated with PC scores used in Fig. 5.

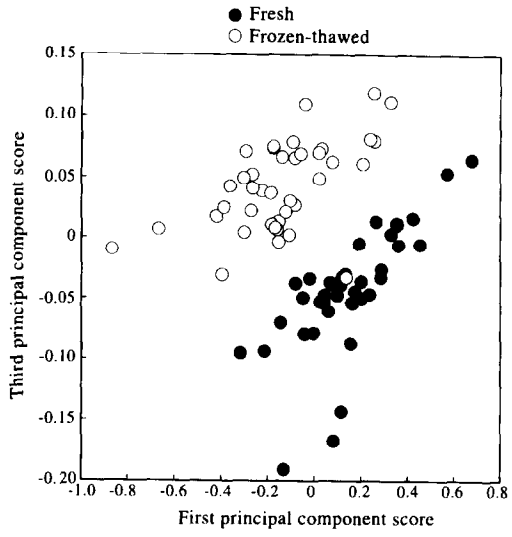

Fig. 7. First and second principal components scores plot for fresh and frozen–thawed chicken.

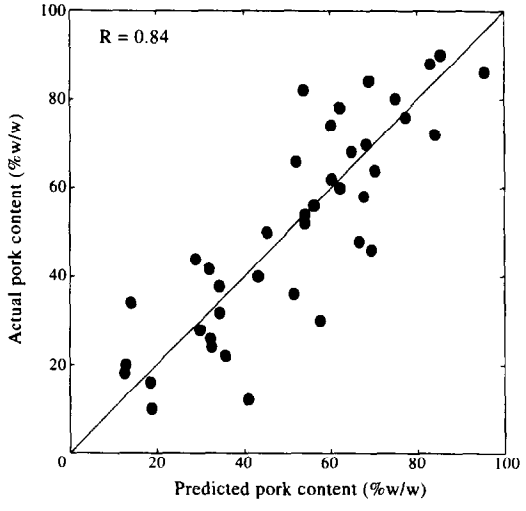

Fig. 8. PLS internal cross-validation results (predicted versus actual pork content) for mixtures of chicken and pork. *R* is the Pearson's correlation coefficient between the predicted and actual values. The standard deviation of the residuals in this case is 13.8% (w/w).

The cross-validation PLS results for the quantitative analysis of turkey or pork in chicken are shown in Figs 8 and 9. In both cases, a trend with increasing turkey or pork meat content is clearly observed. However, there is a considerable degree of scatter which may well arise from mixing difficulties, bearing in mind the relatively small sample volumes interrogated by the infrared beam.

CONCLUSION

Under the very idealised conditions of this experiment, we have been able to show that mid-infrared spectroscopy has the potential to differentiate between selected lean meats. Although we have taken care not to over-fit the models used, we have not used an independent test set in a full discriminant analysis and, therefore, the results cannot be considered conclusive. Similarly, the

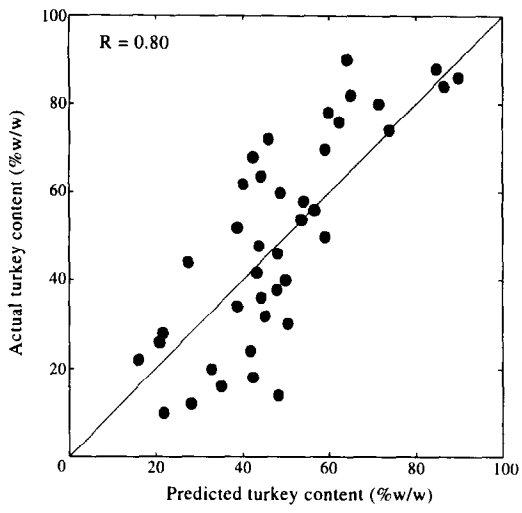

Fig. 9. PLS internal cross-validation results (predicted versus actual turkey content) for mixtures of chicken and turkey. *R* is the Pearson's correlation coefficient between the predicted and actual values. The standard deviation of the residuals in this case is 14.8% (w/w).

results obtained for the fresh versus frozen–thawed meats are encouraging but are not fully validated. We have shown that semi-quantitative estimation of meat mixture composition is feasible.

A great deal more work will have to be performed before the technique could be properly established as a candidate for meat authentication, although it is clear that the potential exists. The detection of adulterated meat products would require the collection of a very large spectral database of many different meats and potential adulterants, and is a major undertaking. However, the present work has succeeded in showing the range of information that can be extracted from the mid-infrared spectra of fresh and frozen–thawed meats.

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