Use of Pyrolysis Mass Spectrometry with Supervised Learning for the Assessment of the Adulteration of Milk of Different Species

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Binary mixtures of 0–20% cows' milk with ewes' milk, 0–20% cows' milk with goats' milk, and 0–5% cows' milk with goats' milk were subjected to pyrolysis mass spectrometry (PyMS). For analysis of the pyrolysis mass spectra so as to determine the percentage adulteration of either caprine or ovine milk with bovine milk, partial least-squares regression (PLS), principal components regression (PCR) and fully interconnected feed-forward artificial neural networks (ANNs) were studied. In the latter case, the weights were modified by using the standard back-propagation algorithm, and the nodes used a sigmoidal squashing function. It was found that each of the methods could be used to provide calibration models which gave excellent predictions for the percentage adulteration with cows' milk to <1% for samples, with an accuracy of ±0.5%, on which they had not been trained. Scaling the *individual* nodes on the input layer of ANNs significantly decreased the time taken for the ANNs to learn, compared with scaling across the whole mass range; however in one case this approach resulted in poor generalization for the estimates of percentage cows' milk in ewes' milk. To assess whether the calibration models had learned the differences between the milk species or the differences due to the different fat content of in each of the milk types, we also analyzed pure milk samples varying in fat content by PyMS. Cluster analysis showed unequivocally that the major variation between the different milk species was not due to variable fat content. Since any biological material can be pyrolyzed in this way, the combination of PyMS with supervised learning constitutes a rapid, powerful, and novel approach to the quantitative assessment of food adulteration generally.

Index Headings: **Authentication; Chemometrics; Neural networks; Pyrolysis mass spectrometry; Quantitative analysis.**

INTRODUCTION

The production of ewes' and goats' milk has gained significant economic importance in certain Mediterranean countries as a result of widespread acceptance of traditional cheeses. Substitution of expensive ewes' or goats' milk with cheaper cows' milk for greater profit within cheese manufacture is an ongoing problem and needs to be tightly controlled. However, the adulterants are sometimes so similar in appearance, taste, and biochemical composition that routine identification of the substitution remains a problem. There is therefore a need to develop accurate, rapid, automated analytical methods for the detection of the adulteration of ovine and caprine milk with bovine milk.

Several methods are currently used for milk species identification. These methods fall into three areas: (1) chromatographic techniques that include gas-liquid chromatography[1,2] and high-performance liquid chromatography;[3,4] (2) electrophoretic methods including gel electrophoresis[5,6] and isoelectric focusing;[7,8] and (3) immunological-based methods such as agar-gel immunodiffusion,[8–10] immunoelectrophoresis,[11] immunodotting,[12] haemagglutination,[13] and various methods based on the enzyme-linked immunosorbent assay (ELISA).[14–16]

Pyrolysis mass spectrometry (PyMS) is a rapid, automated, instrument-based technique that permits the acquisition of spectroscopic data from 300 or more samples per working day. Pyrolysis is the thermal degradation of a complex nonvolatile material in an inert atmosphere or a vacuum. It causes molecules to cleave at their weakest points to produce smaller, volatile fragments called pyrolysate.[17] Curie-point pyrolysis is a particularly reproducible and straightforward version of the technique, in which the sample, dried onto an appropriate metal, is rapidly heated (0.5 s is typical) to the Curie point of the metal, which may itself be chosen (358, 480, 510, 530, 610, and 770 °C are common temperatures). For the analysis of biological material, the usual pyrolysis temperature employed is 530 °C because it has been shown[18,19] to give a balance between fragmentation from polysaccharides (carbohydrates) and protein fractions. A mass spectrometer can then be used to separate the components of the pyrolysate on the basis of their mass-to-charge ratio (m/z) to produce a pyrolysis mass spectrum,[20] which can then be used as a "chemical profile" or fingerprint of the complex material analyzed.

Within the food industry, PyMS has been exploited to confirm the provenance of orange juice[21] and the quality of scotch whisky.[22,23] However, the interpretation of the PyMS spectra has conventionally been by the application of the "unsupervised" pattern recognition methods of principal components analysis (PCA), canonical variates analysis (CVA), and hierarchical cluster analysis (HCA). With "unsupervised learning" methods of this sort, the relevant multivariate algorithms seek "clusters" in the data,[24] thereby allowing the investigator to group objects together on the basis of their perceived closeness; this process is often subjective because it relies upon the interpretation of complicated scatter plots and dendrograms. In addition, such methods, although in some sense quantitative, are better seen as qualitative since their chief purpose is merely to *distinguish* objects or populations.

More recently, various related but much more powerful methods, most often referred to within the framework of chemometrics, have been applied to the "supervised" analysis of PyMS data.[25,26] Arguably, the most significant of these is the application of (artificial) neural networks

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(ANNs). The first demonstration of the ability of ANNs to discriminate between biological samples from their pyrolysis mass spectra was for the qualitative assessment of the assessment of the adulteration of extra virgin olive oils with various seed oils;[27,28] in this study, which was performed double-blind, neural networks were trained with the spectra from 12 virgin olive oils, coded 1 at the output node, and with the spectra from 12 adulterated oils, which were coded 0. This approach permitted their rapid and precise assessment, a task which previously was labor-intensive and very difficult. It was most significant that the traditional ''unsupervised'' multivariate analyses of PCA, CVA, and HCA failed to separate the oils according to their virginity or otherwise but rather discriminated between them on the basis of their cultivar. Several studies have now shown that this combination of PyMS and ANNs is also very effective for the rapid identification of a variety of bacterial strains.[29–32]

The above studies all exploited ANNs to solve classification problems, which by definition are essentially qualitative in nature. However, perhaps the most significant application of ANNs to the analysis of PyMS data is to gain accurate and precise *quantitative* information about the chemical constituents of microbial (and other) samples. For example, it has been shown that it is possible with the use of this method to follow the production of indole in a number of strains of *E. coli* grown on media incorporating various amounts of tryptophan,[33] to quantify the (bio)chemical constituents of complex biochemical binary mixtures of proteins and nucleic acids in glycogen, and to measure the concentrations of tertiary mixtures of cells of the bacteria *Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus*.[34–36] The later study[36] also demonstrated that other supervised learning methods such as partial least-squares (PLS) and principal components regression (PCR) could also be used to extract quantitative information from the spectra of the binary and tertiary mixtures. Finally, the combination of PyMS and ANNs also has potential in the screening and analysis of microbial cultures producing recombinant proteins[37] and antibiotics.[38,39]

The objective of the present study is to demonstrate that the combination of PyMS with multivariate data analyses of PCR, PLS, and ANNs (which employ supervised learning algorithms) can permit the rapid assessment of the *quantitative* adulteration of ovine and caprine milk with bovine milk.

EXPERIMENTAL

Preparation of either Goats' or Ewes' Milk Samples Adulterated with Cows' Milk. Three types of milk of certain provenance were used in this study: cows' milk was purchased from Highmead Dairies, Llanbydder, Dyfed, U.K.; goats' milk from Tipi Dairy Goat, Ammanford, Dyfed, U.K.; and ewes' milk from C. B. Williams & Sons, Towcester, Northants, U.K.

Three mixtures were prepared: (A) the first contained ewes' milk adulterated with 0–20% cows' milk in steps of 1%, while another mixture (B) was of goats' milk adulterated with 0–20% cows' milk in steps of 1%. For both A and B, two sets of mixtures were then prepared; the training set consisted of x % cows' milk and y % ewes' or goats' milk, where x:y were 0:100, 2:98, 4:96, 6:94, 8:92, 10:90, 12:88, 14:86, 16:84, 18:82, and 20:80. The second, ''unknown'' test set consisted of (x:y) 1:99, 3:97, 5:95, 7:93, 9:91, 11:89, 13:87, 15:85, 17:83, and 19:81. The third mixture (C) was of goats' milk adulterated with 0–5% cows' milk in steps of 0.25%. For this experiment two sets of mixtures were also prepared. The training set consisted of x % cows' milk and y % goats' milk, where x:y were 0:100, 0.5:99.5, 1:99, 1.5:98.5, 2:98, 2.5:97.5, 3:97, 3.5:96.5, 4:96, 4.5:95.5, and 5:95. The second, ''unknown'' test set consisted of (x:y) 0.25:99.75, 0.75:99.25, 1.25:98.75, 1.75:98.25, 2.25:97.75, 2.75:97.25, 3.25:96.75, 3.75:96.25, 4.25:95.75, and 4.75:95.25.

Preparation of Cows', Goats', and Ewes' Milk with Differing Fat Contents. In addition to the adulterated samples (detailed above), various mixtures of the three *pure* milks were prepared, which differed in their fat content. Ten-milliliter samples of each milk were centrifuged at 3000 g for 20 min; this process had the desired effect of making the cream layer, which contains the lipids, rise to the top. The thickness of this layer was measured, and the ratio of cream to total milk was calculated (Table IA). Only the fat content of the cows' milk was given (on the container it was supplied in), as 3.8% (±0.1%); this percentage is very similar to the average fat content of cows' milk (from many breeds) stated in 1980 as being 3.86%.[40] The cream:total ratio was then used to find the percentage fat content in the goats' and ewes' milk samples, which were calculated to be 4.3% and 5.4% respectively; these values were also close to the expected published fat contents of 4.5% for goats' milk[41] and 5–7% for ewes' milk.[42] The cream and water layers were then collected separately and mixed to make a range of percentages of fat in the three milk mixtures, details of which are given in Table IB.

Pyrolysis Mass Spectrometry. Two and a half microliters of the above materials was evenly applied onto iron–nickel foils to give a thin, uniform surface coating. Prior to pyrolysis, the samples were oven-dried at 50 °C for 30 min. Each sample was analyzed in triplicate. The pyrolysis mass spectrometer used was the Horizon Instruments PYMS-200X (Horizon Instruments Ltd., Heathfield, E. Sussex, U.K.); for full operational procedures see Refs. 31, 36, and 39. The sample tube carrying the foil was heated, prior to pyrolysis, at 100 °C for 5 s. Curie-point pyrolysis was at 530 °C for 3 s, with a temperature rise time of 0.5 s. The data from PyMS were collected over the m/z range 51 to 200 and may be displayed as quantitative pyrolysis mass spectra (e.g., as in Fig. 1). The abscissa represents the m/z ratio, while the ordinate contains information on the ion count for any particular m/z value ranging from 51 to 200. Data were normalized as a percentage of total ion count to remove the most direct influence of sample size *per se.*

Principal Components Analysis. Principal components analysis is a multivariate statistical technique that can be used to identify *correlations* among a set of variables (in this case 150 m/z intensities) and to transform the original set of variables to a new set of *uncorrelated* variables called principal components (PCs). For the present purpose, PCA can be thought of as finding a set of orthogonal axes in 150-dimensional space; these new

TABLE I. Fat content of cows', goats' and ewes' milk (A) and (B) mixtures of varying fat content.

A

	Cow	Goat	Ewe
Height of liquid (mm)	8.3	8.3	8.3
Cream thickness (mm)	0.7	0.8	1
Ratio of cream to total	0.08	0.10	0.12
Percentage fat[a]	3.8	4.3	5.4

B

	Percentage fat in milk mixtures		
Identifier for CVA plots	Cow	Goat	Ewe
a	0	0	0
b	0.38	0.43	0.54
c	0.76	0.86	1.08
d	1.14	1.29	1.62
e	1.52	1.72	2.16
f	1.9	2.15	2.7
g	2.28	2.58	3.24
h	2.66	3.01	3.78
i	3.04	3.44	4.32
j	3.42	3.87	4.86
k	3.8	4.3	5.4

[a] This percentage was calculated by using the ratio of the volume of cream to total liquid and adjusting so that the cow milk had a fat content of 3.8% (this is the value adhered to ±0.1%).

axes (or PCs) are *linear* combinations of the original variables and are derived in decreasing order of importance; therefore, the first PC accounts for the maximum variation among the samples, and subsequent PCs are chosen to account for progressively decreasing variance.[24,43–48]

The objective of PCA is to see whether the first few PCs account for most of the variation in the original data. If they do reduce the number of dimensions required to display the observed relationships, then the PCs can be plotted, and "clusters" or "trends" may be found in the data. PCA is a variable-directed technique and therefore does not use any *a priori* knowledge of the groupings within samples (objects) in the data set. That is to say, it is unsupervised; thus plots of PCs are thought to display the natural relationships between the samples.

To effect PCA, we processed the normalized data with the GENSTAT package[49] run under Microsoft DOS 6.22 on an IBM-compatible PC; this method has been previously described.[44]

Canonical Variates Analysis. Canonical variates analysis is also a multivariate statistical technique, here carried out with the use of the GENSTAT package. Before CVA was employed, PCA was used to reduce the dimensionality of the data, and only those PCs whose eigenvalues accounted for more than 0.1% of the total variance were used. After the first few PCs, the axes generated will usually be due to random "noise" in the data; these PCs can be ignored without reducing the amount of useful information representing the data, since each PC is now independent of (uncorrelated with) any other PC.

CVA then separated the objects (samples) into groups on the basis of the retained PCs and the *a priori* knowledge of the appropriate number of groupings;[50,51] this step is achieved by minimizing the within-group variance and maximizing the between-group variance. The *a priori* groups here are the known triplicate pyrolysis mass spectra and so do not bias the analysis in any way.

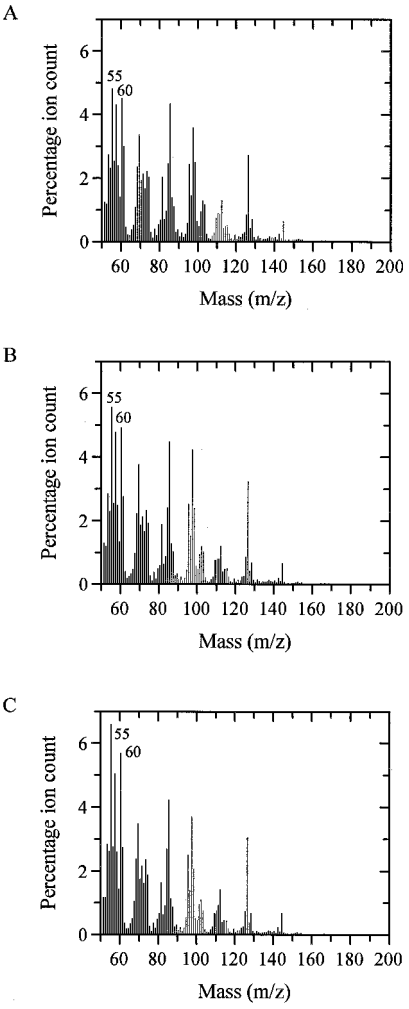

FIG. 1. Representative pyrolysis mass spectra of (**A**) pure cows' milk (**B**) pure goats' milk, and (**C**) pure ewes' milk.

The principle of CVA is similar to that of PCA, but because the objective of CVA is to maximize the ratio of the between-group to within-group variance, a plot of the first two canonical variates (CVs) displays the best two-dimensional representation of the *group* separation.

Artificial Neural Networks. All ANN analyses were carried out under Microsoft Windows NT 3.51 on an IBM-compatible PC. Data were normalized prior to analysis with the use of the Microsoft Excel 4.0 spreadsheet. The back-propagation (BP) neural network simulation program used was NeuralDesk (Neural Computer Sciences, Southampton, Hants, U.K.) as previously described in Refs. 31, 36, and 52.

The structure of the ANN used in this study to analyze pyrolysis mass spectra consisted of three layers containing 159 processing nodes (neurons or units) made up of the 150 input nodes (normalized averaged pyrolysis mass spectra), 1 output node (percentage milk adulteration), and one "hidden" layer containing 8 nodes (i.e., a 150–8–1 architecture; see Fig. 2 for a diagrammatic representation). Each of the 150 input nodes was connected to the 8 nodes of the hidden layer by using abstract interconnections (connections or synapses). Each connec-

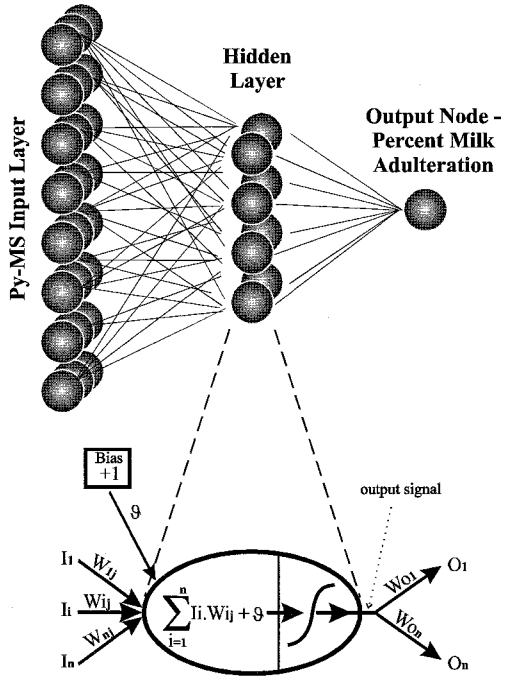

FIG. 2. A neural network consisting of 24 inputs (data for PyMS actually consisted of 150 inputs/masses) and 1 output node (which represented the percentage cow milk adulterated in goat or sheep milk) connected to each other by 1 hidden layer consisting of 8 nodes. In the architecture shown, adjacent layers of the network are fully interconnected, although other architectures are possible. One of the nodes in the hidden layer is given in more detail, showing the information processing by node. An individual node sums its input (the Σ function) from nodes in the previous layer, including the bias (θ), transforms them via a ''sigmoidal'' squashing function, and outputs them to the next node to which it is linked via a connection weight.

tion has an associated real value, termed the weight, that scales signals passing through it. Nodes in the hidden layer sum the signals feeding to them and output this sum to each driven connection scaled by a ''squashing'' function (f) with a sigmoidal shape, the function $f = 1/(1 + e^{-x})$, where $x = \Sigma$inputs. These signals are then passed to the output node, which sums them; in turn, squashed by the sigmoidal activation function, the product of this node is then feed to the ''outside world''.

In addition, the hidden layer and output node were connected to a bias (whose activation was always set to +1), making a total of 1217 connections, whose weights were altered during training. Before training commenced, the values applied to the input and output nodes were normalized between 0 and +1; the input layer was scaled so that the lowest ion count was set to 0 and the highest to 1 either (1) globally across the *whole* mass range or (2) for each input mass. Finally, the connection weights were set to small random values (typically between −0.005 and +0.005).

The algorithm used to train the neural network was the standard back-propagation.[53–56] For the training of the ANN, each input (i.e., normalized averaged pyrolysis mass spectrum) is paired with a desired output (i.e., the percentage cows' milk in either goats' or ewes' milk, the determinand); together these are called a training pair (or training pattern). An ANN is trained over a number of training pairs; this group is collectively called the training set. The input is applied to the network, which is allowed to run until an output is produced at each output node. The differences between the actual and the desired output, taken over the entire training set, are fed back through the network in the reverse direction to signal flow (hence back-propagation), modifying the weights as they go. The weights were updated in the batch mode, while the 11 training patterns were presented in a random order. This process is repeated until a suitable level of error is achieved. In the present work, a learning rate of 0.1 and a momentum of 0.9 were used.

Each epoch represented 1217 connection weight updatings and a recalculation of the root mean square (rms) error between the true and desired outputs over the entire training set [rms error of formation (RMSEF)]. During training a plot of the error vs. the number of epochs represents the ''learning curve'' and may be used to estimate the extent of training. Training may be said to have finished when the network has found the lowest error. Provided that the network has not become stuck in a local minimum, this point is referred to as the global minimum on the error surface.

It is known[33,36,53,54,57] that neural networks can become over-trained. An over-trained neural network has usually learned the stimulus patterns it has seen perfectly but cannot give accurate predictions for unseen stimuli; i.e., it is no longer able to generalize. For ANNs accurately to learn and predict the concentrations of determinands in biological systems, networks must obviously be trained to the correct point. Therefore, during training the network was also interrogated with the test set, and the rms error between the output seen and that expected was calculated [rms error of prediction (RMSEP)]; training was stopped when the error on the cross-validation data was lowest.

Finally after training, all pyrolysis mass spectra of the three milk mixtures were used as the ''unknown'' inputs (test data); the network then output its estimate (best fit) in terms of the percentage cows' milk adulteration in either goat's or ewes' milk.

Principal Components Regression and Partial Least-Squares. All PCR and PLS analyses were carried out by using the program Unscrambler II Ver. 4.0 (CAMO A/S, Trondheim, Norway) (and see Ref. 48), which runs under Microsoft MS-DOS 6.2 on an IBM-compatible PC. Data were also processed prior to analysis by using the Microsoft Excel 4.0 spreadsheet, run under Microsoft Windows NT on an IBM-compatible PC.

The first stage was the preparation of the data. This step was achieved by presenting the ''training set'' as two data matrices to the program: **X**, which contains the normalized averaged pyrolysis mass spectra, and **Y**, which represents the percentage of the determinand (i.e., cows' milk). Unscrambler II also allows the addition of ''start noise'' (i.e., noise to the **X** data); this option was not used. Finally, the **X** data were mean-centered and scaled in proportion to the reciprocal of their standard deviations.

The next stage was the generation of the calibration model; this procedure first requires the user to specify the appropriate algorithm. The Unscrambler II program has one PCR algorithm and two PLS algorithms: PLS1, which handles only one Y variable at a time, and PLS2, which will model several Y variables simultaneously.[48]

Since only one Y variable was to be predicted, the PCR and PLS1 algorithms were used.

The method of validation used was full cross-validation, via the leave-one-out method.[48] This technique sequentially omits one sample from the calibration; the PCR or PLS model is then re-determined on the basis of this reduced sample set. The percentage of milk adulteration of the omitted sample is then predicted with the use of the model. This method is required to determine the optimal size of the calibration model, in order to obtain good estimates of the precision of the multivariate calibration method (i.e., to neither under- nor over-fit predictions of unseen data).[48,58–60] Unscrambler also has reasonably sophisticated outlier detection methods; although these were employed, it was not necessary to delete any of the objects from the calibration models formed.

For selection of the optimal number of principal components or PLS factors to use in predictions after the model was calibrated, the rms error between the true and desired percentage adulteration over the entire calibration model was calculated for the known training set (RMSEF) and unknown mass spectra (RMSEP). These rms errors were then plotted against the number of latent variables (factors) used in predictions. With the use of this approach, after calibration, to choose the optimal number of PCs or PLS factors to use in the prediction, we used all pyrolysis mass spectra as the ''unknown'' inputs (test data); the model then gave its prediction in terms of the percentage milk adulteration.

RESULTS AND DISCUSSION

Pyrolysis mass spectra of pure cows', goats', and ewes' milk are shown in Fig. 1. With the exception of the *m*/*z* peaks at 55 and 60, there was little qualitative difference between these spectra; small quantitative differences between the spectra from the different milk species were observed. Such spectra readily illustrate the need to employ multivariate statistical techniques in the analysis of PyMS data.

The objective of the present study was to quantify the contamination of either goats' or ewes' milk with cows' milk. It is at least plausible that the pyrolysis mass spectra of nonvolatile complex bio-materials will be dominated by masses attributed to their lipids; this is because these species have *relatively* low volatility and are therefore preferentially vaporized when subjected to pyrolysis. To assess whether the subtle spectral differences between the three milk species analyzed, as observed in Fig. 1, were due only to variable fat content, rather than other biochemical characteristics (e.g., such as protein and carbohydrate content), we analyzed pure milk samples that varied in fat content alone by PyMS.

Mixtures of the three *pure* milks that differed in their fat content were therefore prepared (see Table I for details) and analyzed by PyMS. For observation of the relationship between these 33 different samples, each represented by triplicate spectra, each was coded, by using this *a priori* knowledge about which were replicates, to give 33 individual groups (see Table I) and then analyzed by canonical variates analysis. The resulting CVA plot is shown in Fig. 3, where the first, second, and third CVs accounted for 76.7, 10.8, and 6.5% (94.0% total) of the total variance, respectively. It can be observed that three clusters are formed, which are representative of the different milk species irrespective of the different fat contents analyzed. It was therefore obvious that the major quantitative differences in the *m*/*z* values seen in the mass spectra of the pure milks (Fig. 1) were not due solely to lipid content; protein, carbohydrate, and other biochemical species were also important. It is noteworthy that increasing fat content in the milks can also be observed in the CVA plot (Fig. 3); arrows have been drawn on this CVA plot that show this feature to be a combination of the first and second CV. Other studies have shown that it is also possible to use PyMS to measure the fat content in cows', goats', and ewes' milk; however these findings are not relevant here and will be elaborated elsewhere (Goodacre and Kell, paper in preparation).

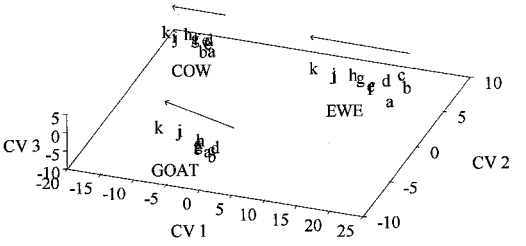

FIG. 3. Pseudo 3-D CVA plot based on PyMS data analyzed by GENSTAT, showing the relationship between the three types of milk with varying fat content. The first, second, and third CVs accounted for 76.7, 10.8, and 6.5% (94.0% total) of the total variance, respectively. The identity and fat content of the milk samples are given in Table I. The arrows indicate increasing fat content.

Quantification of the Adulteration of Ewes' Milk with Cows' Milk. After the collection of the pyrolysis mass spectra of 0–20% cows' milk (in 1% steps) in ewes' milk, the first stage was to look at the relationship between the pyrolysis mass spectra of these mixtures by using principal components analysis. PCA is a well-known method for reducing the dimensionality of multivariate data while preserving most of the variance;[24,43,46,48] in our pyrolysis mass spectral data this reduction will be from the 150 *m*/*z* values to two principal components. The first and second PCs are plotted in Fig. 4 and accounted for 91.6 and 5.4% (96.9% total) of the total variance, respectively. It was evident that neither PC alone nor a combination of PC 1 and PC 2 could simply account for or describe the difference in the amount of cows' milk in ewes' milk. When other PCs were plotted (data not shown), these also failed to account for the adulteration; this result was perhaps not surprising given the large amount of variance (96.9%) in the first two PCs. It is likely that PCA was unsuccessful because it relies on ''unsupervised'' learning and *linear* (orthogonal) transformations of the raw multivariate data and therefore cannot provide the truly best analytical discriminations. A more elegant approach is to use ''supervised'' learning methods that employ nonlinear algorithms.

Therefore ANNs were trained, by using the standard back-propagation algorithm, with the 11 normalized averaged PyMS data from the training sets as the inputs, scaled across the *whole* mass range so that the lowest mass intensity was set to 0 and the highest mass intensity to 1, and the percentage cows' milk adulteration (0–20%) as the output, the latter being scaled between 0 and 20.

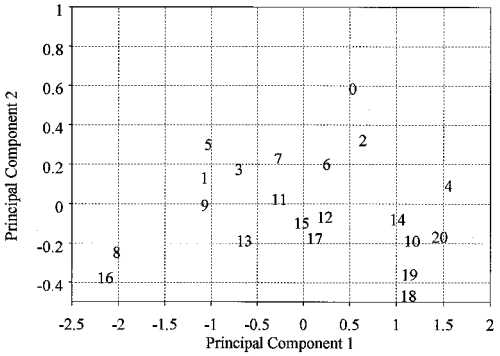

FIG. 4. PCA plot based on PyMS data analyzed by GENSTAT, showing the relationship between ewes' milk adulterated with between 0 to 20% cows' milk. The first and second PCs accounted for 91.6 and 5.4% (96.9% total) of the total variance, respectively. The numbers refer to the percentage adulteration.

The effectiveness of training was expressed in terms of the rms error between the actual and desired network outputs, and during training the network was interrogated with the test set of 10 pyrolysis mass spectra. These rms errors were used to detect overtraining; that is to say, the error in the training set decreases, but the error in the test set increases. It is important not to over-train ANNs since (by definition) the network will not generalize well.[33,36,53,54,57] It was found that the minimum rms error in the test set (1.21) was reached when the rms error of the training set was 0.02 and optimal training had occurred; this took approximately $6{\cdot}10^4$ epochs (Table II). The ANN was then interrogated with the training and test sets, and a plot of the network's estimate vs. the true percentage of cows' milk (Fig. 5) gave a linear fit (bold line) that was very close to the expected proportional fit (i.e., $y = x$; shown here as a broken line). It was therefore evident that the network's estimate of the quantity of cows' milk adulteration in the mixtures was very similar to the true quantity, both for spectra that were used as the training set and, most importantly, for the "unknown" pyrolysis mass spectra.

In other studies ANNs were set up with the standard BP algorithm with the same architecture as the one used above, except that the input layer was scaled for *each* input node so that the lowest mass was set to 0 and the highest mass to 1. The network was still able to converge, but took only 10^2 epochs, compared with $6{\cdot}10^4$ used above. However, although these ANNs trained 600 times faster, they did not generalize as well; after training with the same test set cross-validation method detailed above, the rms error in the training set was 0.23% and the rms error in the test set was now 2.98, compared with 1.21 obtained previously (Table II). ANNs of both topologies were trained three times, with different random starting weights, and the same generalization point was found. This result was reproducible and therefore not due to the random starting weights' being set by chance to values very close to the weights set by the ANNs at the best generalization point. In a procedure to assess the precision of the PyMS technique, ANNs were trained and interrogated with the triplicate normalized spectra, and the precision in terms of a pooled standard deviation was found to be 0.9 and 1.9 respectively, for the training and test sets, (the overall standard deviation for all the data was 1.4).

TABLE II. Comparison of the artificial neural network calibration with partial least-squares, principal components regression, and multiple linear regression in the deconvolution of pyrolysis mass spectra for determining the percentage volume of cow's milk in the range 0 to 20% mixed in ewes' milk.

Root mean square error between true values and estimates of the percentage of ewe's milk adulteration[a]	ANNs[b]	ANNs[c]	PLS	PCR	MLR
No. epochs[d]/factors	60,000	100	4	7	—
RMSEF	0.02	0.23	0.22	1.30	0.00
RMSEP	1.21	2.98	2.77	2.83	2.90
For the estimates from PyMS in the test set					
Slope	0.90	0.89	0.58	0.55	0.58
Intercept	0.63	−0.85	3.30	3.85	3.02
Correlation coefficient	0.98	0.92	0.95	0.96	0.95

[a] The comparison is of the optimal calibration models as judged by test set cross-validation.
[b] The input layer was scaled across the *whole* mass range so that the lowest mass was set to 0 and the highest mass to 1.
[c] The input layer was scaled for *each* input node so that the lowest mass was set to 0 and the highest mass to 1.
[d] Calculated by taking the average of three training sessions.

In further studies, other "supervised" learning methods that employ multivariate linear regression, such as partial least squares, principal components regression, and multiple linear regression (MLR), were also applied with the use of the same training and test sets as used for the ANN analyses. Again, test set cross-validation was used so as to assure that the calibration models constructed by PCR and PLS were not over-fitting. Table II also gives the rms error for the predictions produced by MLR, PCR, and PLS on both the training and test sets for 0–20% cows' milk mixed in ewes' milk. Also detailed in this table are the slopes and intercepts of the best-fit lines and correlation coefficient for these three linear methods compared with the ANN analyses. It can be seen that there is very little difference between the predictive power of MLR, PCR and PLS, although PLS still performs best with a test set error of 2.77, compared with 2.83 and 2.90 for PCR and MLR, respectively. In addition, these

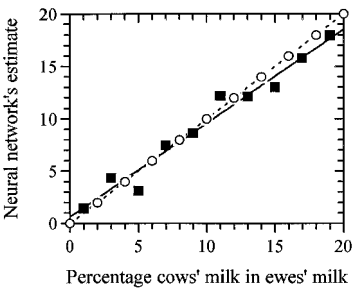

FIG. 5. The estimates of trained 150–8–1 neural networks vs. the true percentage volume of cows' milk (0–20%) in ewes' milk. The input layer was scaled across the *whole* mass range so that the lowest mass was set to 0 and the highest mass to 1; the networks were trained by using the standard back-propagation algorithm, for approximately 60,000 epochs, to the point given in Table II by test set cross-validation. Open circles represent spectra that were used to train the network, and closed circles indicate "unknown" spectra that were not in the training set. The calculated linear fit (bold line) and expected proportional fit (broken line) are shown.

TABLE III. Comparison of artificial neural network calibration with partial least-squares, principal components regression, and multiple linear regression in the deconvolution of pyrolysis mass spectra for determining the percentage volume of cows' milk in the range 0 to 20% mixed in goats' milk.

Root mean square error between true values and estimates of the percentage of goats' milk adulteration					
	ANNs[b]	ANNs[c]	PLS	PCR	MLR
No. epochs[d]/factors	4000	100	6	6	—
RMSEF	1.20	0.24	0.07	0.70	0.00
RMSEP	2.02	1.80	1.47	1.44	1.46
For the estimates from PyMS in the test set					
Slope	0.99	0.95	1.01	0.97	1.01
Intercept	−0.40	−0.12	−0.57	−0.16	−0.57
Correlation coefficient	0.95	0.96	0.97	0.97	0.97

[a] The comparison is of the optimal calibration models as judged by test set cross-validation.
[b] The input layer was scaled across the *whole* mass range so that the lowest mass was set to 0 and the highest mass to 1.
[c] The input layer was scaled for *each* input node so that the lowest mass was set to 0 and the highest mass to 1.
[d] Calculated by taking the average of three training sessions.

linear regression methods gave very similar results to ANNs where the input layer was scaled for *each* input node. ANNs where the input layer was scaled across the *whole* mass range, however, gave best results by a factor of 2 as judged by the RMSEPs.

Scaling *each* input node will give each mass equal weight for formation of the ANN model; in contrast, scaling *across* the mass range results in the most intense masses having most influence over smaller masses. The fact that the latter models gave best results implies that there was some noise in the mass spectral data (particularly in *m/z* values of low intensity) to which the ANNs scaled across the input mass range were robust but which the linear regression analyses and ANNs, where each input was scaled individually, must have incorporated into their calibration models. Furthermore, it has previously been observed that a small amount of noise in pyrolysis mass spectral data,[36] to which ANNs were robust but which PLS and PCR incorporated into their calibration models, gave less accurate predictions for the determinand in three sets of binary mixtures.[36] It is likely that if the masses which contribute noise in the PyMS spectra were removed, then PLS, PCR, and ANNs (scaled for *each* input node) would give more accurate estimates of ewes' milk adulteration with cows' milk. Indeed, variable selection methods have been applied to these data, and the removal of masses contributing to noise has resulted in better predictive power. However, although significant, this observation will be reported elsewhere (Goodacre et al., paper in preparation).

Quantification of the Adulteration of Goats' Milk with Cows' Milk. Samples of 0–20% cows' milk (in 1% steps) in goats' milk were prepared and the 21 mixtures analyzed in triplicate by PyMS. As detailed above, the normalized averaged spectra were then split in the training set (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20% cows' milk) and test set (1, 3, 5, 7, 9, 11, 13, 15, 17, and 19%) and analyzed by MLR, PCR, PLS, and two ANNs models where the input nodes were scaled either across the mass range or individually.

Table III gives the rms error for the predictions produced by each of the five methods above on both the training set (RMSEF) and test set (RMSEP) for 0–20% cows' milk mixed in goats' milk; also detailed in this table are the slopes and intercepts of the test set best-fit lines and correlation coefficient for the test set. It can be observed that all the linear regression methods gave very similar predictions, and the RMSEPs were 1.47 for PLS, 1.44 for PCR, and 1.46 for MLR. As observed above, the ANNs scaled for each mass input trained very quickly as usual[35]—typically 10^2 epochs, compared with 4.10^3 epochs for ANNs (40 times quicker) where the input layer was scaled across the mass range to lie between 0 and 1. By contrast to results observed when quantifying cows' milk in ewes' milk, the predictive power of ANNs scaled individually on the input layer was slightly better; it was 2.02 RMSEP compared with 1.80 for ANNs scaled across the mass range. Although all five methods gave very similar results, the predictions of the ANNs were in fact worse than those for MLR, PCR, and PLS. ANNs were also trained and interrogated with the triplicate normalized spectra, and the precision in terms of a pooled standard deviation was found to be 0.5 and 2.5, respectively, for the training and test sets (the overall standard deviation for all the data was 1.5).

The use of PLS and PCR for the deconvolution of spectroscopic data is well documented.[48] Indeed, studies comparing multiple least-squares methods as well as the latent variable PCR and PLS methods[61–63] have concluded that the best technique appears to be PLS. Other studies[36,39,64–67] have concluded that ANNs often give better predictions than does PLS because ANNs are able to perform nonlinear mappings of the inputs to output(s) while still being able to map the linear ones.

The estimates of calibrated PLS models vs. the true percentage volume of cows' milk (0–20%) in goats' milk are shown in Fig. 6. It can be observed that the PLS model's estimate of the quantity of cows' milk adulteration in mixtures with goats' milk was indeed very similar to the true quantity, for spectra that were used as both the training and test sets. As detailed above, test set cross-validation was used to find the best calibration model, and this was found to be the case where six latent variables were used. That optimal calibration occurred with the use of >3 latent variables, a phenomenon that has been seen previously,[36,39] which usually implies that there are nonlinear relationships within the pyrolysis mass spectral data. It was therefore rather surprising that PLS gave significantly better results than did either of the ANNs studied on this dataset.

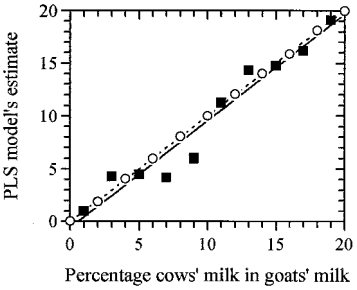

FIG. 6. The estimates of calibrated PLS models vs. the true percentage volume of cows' milk (0–20%) in goats' milk. The best calibration model was formed by using six latent variables and was calculated by test set cross-validation. Open circles represent spectra that were used to form the model, and closed circles indicate "unknown" spectra that were not in the training set. The calculated linear fit (bold line) and expected proportional fit (broken line) are shown.

Lowering the Limit of Detection of Cows' Milk Adulteration in Goats' Milk. The errors for the best predictions, for the test set only, of the adulteration of cows' milk in either ewes' or goats' were 1.21 (Table II; ANNs scaled across mass range) and 1.44 (Table III; PCR), respectively. This result indicates that the overall predictive accuracy of PyMS combined with ANNs was between ±1.2% and ±1.5%. To assess whether PyMS could be used to detect very low levels of adulteration (<1%), we prepared samples containing 0–5% cows' milk (in 0.25% steps) in goats' milk and analyzed them by PyMS.

ANNs were trained with the 11 normalized averaged PyMS data from the training sets as the inputs, scaled for *each* input node so that the lowest mass was set to 0 and the highest mass to 1, and the percentage cows' milk adulteration (0–5%) as the output, the latter being scaled between 0 and 5. Test set cross-validation found that the minimum rms error in the test set was 0.54, and this figure was reached when the rms error of the training set was 0.03, which took approximately $3 \cdot 10^2$ epochs (Table IV). The ANN was then interrogated with the training and test sets, and a plot of the network's estimate vs. the true percentage of cows' milk (Fig. 7) gave a linear fit (bold line), which was indistinguishable form the expected proportional fit (i.e., $y = x$; shown here as a broken line); the slope of the best fit line was 0.99, and the intercept was 0.08. With the exception of the 1.75% cows' milk in goats' milk mixture, the network's estimates for the "unknown" pyrolysis mass spectra in terms of the percentage cows' milk adulteration were very similar to the true quantity. For assessment of the precision of the PyMS technique at this lower adulteration range, ANNs were trained and interrogated with the triplicate normalized spectra, and the precision in terms of a pooled standard deviation was found to be 0.3 and 0.8, respectively, for the training and test sets (the overall standard deviation for all the data was 0.5).

In other studies ANNs were set up by using the standard BP algorithm with the same architecture as the one used above, except that the input layer was scaled across the whole mass range; MLR, PCR, and PLS were also calibrated with test set cross-validation with the same training and test sets as detailed above. Table IV gives the RMSEFs and RMSEPs for 0–5% cows' milk mixed in goats' milk; also detailed in this table are the slopes and intercepts of the test set best-fit lines and correlation coefficient for only the test set. It can be seen that all the calculated RMSEPs were very similar and fall in the range between 0.47 and 0.54. Although the ANN where each input was scaled gives the highest RMSEP (0.54), this calibration model was taken to be the best because the slope and intercept were closest to $y = x$, i.e., 0.99 instead of 1 and 0.08 instead of 0, respectively. Likewise, the other ANNs had significantly better slopes and intercepts than did any of the linear regression methods.

These results show that the predictive accuracy of PyMS combined with supervised learning methods was within ±0.5% when samples containing 0–5% cows' milk in goats' milk were analyzed. It is plausible that the predictive accuracy of ±0.5% was significantly lower than the ±1.2%–±1.5% seen for samples containing 0–20% cows' milk in goats' milk, because more samples per percent were analyzed; that is to say, the 0–20% range contained only two samples per percent, whereas

TABLE IV. Comparison of artificial neural network calibration with partial least-squares, principal components regression, and multiple linear regression in the deconvolution of pyrolysis mass spectra for determining the percentage volume of cows' milk in the range 0 to 5% mixed in goats' milk.

Error between true values and estimates of the percentage of goats' milk adulteration[a]	ANNs[b]	ANNs[c]	PLS	PCR	MLR
No. epochs[d]/factors	50,000	300	4	10	—
RMSEF	0.01	0.03	0.07	0.00	0.00
RMSEP	0.48	0.54	0.47	0.48	0.51
For the estimates from PyMS in the test set					
Slope	0.92	0.99	0.85	0.86	0.84
Intercept	0.14	0.08	0.36	0.35	0.37
Correlation coefficient	0.94	0.94	0.94	0.94	0.94

[a] The comparison is of the optimal calibration models as judged by test set cross-validation.
[b] The input layer was scaled across the *whole* mass range so that the lowest mass was set to 0 and the highest mass to 1.
[c] The input layer was scaled for *each* input node so that the lowest mass was set to 0 and the highest mass to 1.
[d] Calculated by taking the average of three training sessions.

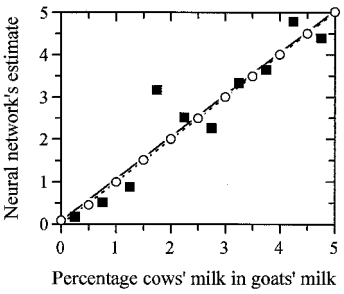

FIG. 7. The estimates of trained 150–8–1 neural networks vs. the true percentage volume of cows' milk (0–5%) in goats' milk. The input layer was scaled for *each* input node so that the lowest mass was set to 0 and the highest mass to 1; the networks were trained by using the standard back-propagation algorithm, for approximately 300 epochs, to the point given in Table II by test set cross-validation. Open circles represent spectra that were used to train the network, and closed circles indicate "unknown" spectra that were not in the training set. The calculated linear fit (bold line) and expected proportional fit (broken line) are shown.

by contrast, the 0–5% range contained five exemplars. Moreover, as illustrated here, by reducing the range to 0–5% and increasing the number of examples used to calibrate the PCR, PLS, or ANN models, one can use PyMS to give excellent predictions for the percentage adulteration with cows' milk to <1% for samples which they had not seen.

CONCLUSION

PyMS and cluster analysis were used unequivocally to classify cows', goats', and ewes' milk irrespective of whether the fat content of the milk varied. It can be concluded from this study that the major quantitative differences in the *m/z* values seen in the mass spectra of the pure milks (Fig. 1) were not due solely to lipid content. Given the very complex nature of milk, this result was not surprising, and it is certain that these ''biochemical fingerprints'' (Fig. 1) also contained a wealth of significant information from protein, carbohydrate, and other biochemical species.

Binary mixtures in the range 0–20% cows' milk with either ewes' or goats' milk were next analyzed by PyMS. ANNs and linear regression techniques (MLR, PCR, and PLS) were employed successfully for the quantitative deconvolution of these pyrolysis mass spectra. It was found that each of the methods could be used to provide calibration models that gave excellent predictions for the percentage adulteration with cows' milk between ±1.2% and ±1.5% for samples for which they had not been trained.

Further experiments were conducted to detect very low levels of adulteration for samples containing 0–5% cows' milk (in 0.25% steps) in goats' milk. The test set predictive accuracy was now significantly better at ±0.5%. Indeed by analyzing this smaller aduleration range, we were able to use PyMS and ANNs to give excellent predictions for the percentage adulteration with cows' milk to <1%.

Scaling the *individual* nodes on the input layer of ANNs significantly decreased the time taken for the ANNs to learn, compared to scaling across the whole mass range. These ANNs trained between 40 to 600 times faster; however, in one case of the three studied, this approach resulted in significantly poorer generalization for the estimates of percentage cows' milk in ewes' milk.

PyMS is a physico-chemical method which has been extensively exploited for whole-organism fingerprinting.[26,68] Other spectroscopic techniques that have also been used for microbial identification include UV resonance Raman spectroscopy[69,70] and Fourier transform infrared spectroscopy (FT-IR).[71,72] These methods all produce complex reproducible biochemical fingerprints that are qualitatively distinct for different samples and quantitative with respect to target determinands. Indeed FT-IR has been exploited recently within the food manufacture industry for the authentication of vegetable oils[73] and fruit purees[74]; furthermore, Raman spectroscopy has also been investigated for the analysis of foods.[75]

The combination of PyMS and ANNs has been shown previously to be an excellent technique capable of the exquisitely sensitive *qualitative* assessment of the adulteration of extra virgin olive oils with various seed oils.[27,28] Here it is shown, for the first time, that PyMS and supervised learning can be used for the *quantitative* assessment of the adulteration of milk. Therefore, in conclusion, since any biological material can be pyrolyzed in this way, the combination of PyMS with supervised learning may be seen to constitute a rapid, powerful, and novel approach to the qualitative *and* quantitative assessment of food adulteration in general.

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1. J. L. Iverson and A. J. Sheppard, J. Dairy Sci. **72,** 1707 (1989).
2. M. J. Pager, J. AOAC **71,** 418 (1989).
3. W. Haasnot, D. P. Venema, and H. L. Elenbaas, Milchwissenschaft **41,** 642 (1986).
4. W. Urbanke, W. Luf, and E. Brandl, Z. Lebensm. Unters. Forsch. **195,** 137 (1992).
5. R. Aschaffenburg and J. E. Dance, J. Dairy Res. **35,** 383 (1968).
6. L. Amigo, M. Ramos, P. J. Martin-Alvarez, and M. Barbosa, J. Dairy Sci. **74,** 1482 (1990).
7. F. Addeo, L. Moio, L. Chianese, and G. Nota, Ital. J. Food Sci. **3,** 71 (1989).
8. L. Amigo, M. Ramos, L. Calhau, and M. Barbosa, Lait **72,** 95 (1992).
9. E. Gombozc, E. Hellwig, and F. Petuely, Z. Lebensm. Unters. Forsch. **172,** 178 (1981).
10. T. García, R. Martín, E. Rodríguez, P. E. Hernández, and B. Sanz, J. Diary Res. **56,** 691 (1989).
11. D. V. Radford, Y. T. Tchan, and J. McPhillips, Aust. J. Dairy Technol. **36,** 144 (1981).
12. P. Aranda, R. Orla, and M. Calvo, J. Dairy Res. **55,** 121 (1988).
13. D. Levieux, Ann. Rech. Vet. **11,** 151 (1980).
14. T. García, R. Martín, E. Rodríguez, J. I. Azcona, B. Sanz, and P. E. Hernández, J. Food Prot. **54,** 366 (1991).
15. E. Rodríguez, R. Martín, T. García, J. I. Azcona, B. Sanz, and P. E. Hernández, J. Int. Food Sci. Technol. **26,** 457 (1991).
16. E. Rodríguez, R. Martín, T. García, I. González, P. Morales, B. Sanz, and P. E. Hernández, J. Sci. Food Agric. **61,** 175 (1993).
17. W. J. Irwin, *Analytical Pyrolysis: A Comprehensive Guide* (Marcel Dekker, New York, 1982).
18. W. Windig, P. G. Kistemaker, J. Haverkamp, and H. L. C. Meuzelaar, J. Anal. Appl. Pyrol. **2,** 7 (1980).
19. R. Goodacre, Ph.D. Thesis, University of Bristol (1992).
20. H. L. C. Meuzelaar, J. Haverkamp, and F. D. Hileman, *Pyrolysis Mass Spectrometry of Recent and Fossil Biomaterials* (Elsevier, Amsterdam, 1982).
21. R. E. Aries, C. S. Gutteridge, and R. Evans, J. Food Sci. **51,** 1183 (1986).
22. K. J. G. Reid, J. S. Swan, and C. S. Gutteridge, J. Anal. Appl. Pyrol. **25,** 49 (1993).
23. R. I. Aylott, A. H. Clyne, A. P. Fox, and D. A. Walker, Analyst **119,** 1741 (1994).
24. B. S. Everitt, *Cluster Analysis* (Edward Arnold, London, 1993).
25. R. Goodacre, Microbiol. Eur. **2,** 16 (1994).
26. R. Goodacre and D. B. Kell, Cur. Opin. Biotechnol. **7,** 20 (1996).
27. R. Goodacre, D. B. Kell, and G. Bianchi, Nature **359,** 594 (1992).
28. R. Goodacre, D. B. Kell, and G. Bianchi, J. Sci. Food Agric. **63,** 297 (1993).
29. J. Chun, E. Atalan, S. B. Kim, H. J. Kim, M. E. Hamid, M. E. Trujillo, J. G. Magee, G. P. Manfio, A. C. Ward, and M. Goodfellow, FEMS Microbiol. Lett. **114,** 115 (1993).
30. R. Freeman, R. Goodacre, P. R. Sisson, J. G. Magee, A. C. Ward, and N. F. Lightfoot, J. Med. Microbiol. **40,** 170 (1994).
31. R. Goodacre, M. J. Neal, D. B. Kell, L. W. Greenham, W. C. Noble, and R. G. Harvey, J. Appl. Bacteriol. **76,** 124 (1994).
32. R. Goodacre, S. J. Hiom, S. L. Cheeseman, D. Murdoch, A. J. Weightman, and W. G. Wade, Cur. Microbiol. **32,** 77 (1996).
33. R. Goodacre and D. B. Kell, Anal. Chim. Acta **279,** 17 (1993).
34. R. Goodacre, A. N. Edmonds, and D. B. Kell, J. Anal. Appl. Pyrol. **26,** 93 (1993).
35. M. J. Neal, R. Goodacre, and D. B. Kell, in *Proceedings of the*

World Congress on Neural Networks, San Diego, California (1994) (International Neural Network Society), p. I318.
36. R. Goodacre, M. J. Neal, and D. B. Kell, Anal. Chem. **66,** 1070 (1994).
37. R. Goodacre, A. Karim, M. A. Kaderbhai, and D. B. Kell, J. Biotechnol. **34,** 185 (1994).
38. R. Goodacre, S. Trew, C. Wrigley-Jones, M. J. Neal, J. Maddock, T. W. Ottley, N. Porter, and D. B. Kell, Biotechnol. Bioeng. **44,** 1205 (1994).
39. R. Goodacre, S. Trew, C. Wrigley-Jones, G. Saunders, M. J. Neal, N. Porter, and D. B. Kell, Anal. Chim. Acta **313,** 25 (1995).
40. M. E. Castle and P. Watkins, *Modern Milk Production* (Faber and Faber, London, 1984).
41. D. MacKenzie, *Goat Husbandry* (Faber and Faber, London, 1980).
42. A. Fraser, *Sheep Production* (Thomas Nelson and Sons, London, 1947).
43. C. Chatfield and A. J. Collins, *Introduction to Multivariate Analysis* (Chapman and Hall, London, 1980).
44. C. S. Gutteridge, L. Vallis, and H. J. H. MacFie, in *Computer-assisted Bacterial Systematics,* M. Goodfellow, D. Jones, and F. Priest, Eds. (Academic Press, London, 1985), p. 369.
45. I. T. Jolliffe, *Principal Component Analysis* (Springer-Verlag, New York, 1986).
46. D. R. Causton, *A Biologist's Advanced Mathematics* (Allen and Unwin, London, 1987).
47. B. Flury and H. Riedwyl, *Multivariate Statistics: A Practical Approach* (Chapman and Hall, London, 1988).
48. H. Martens and T. Næs, *Multivariate Calibration* (John Wiley, Chichester, 1989).
49. J. A. Nelder, *Genstat Reference Manual* (Scientific and Social Service Program Library, University of Edinburgh, Edinburgh, 1979).
50. H. J. H. MacFie, C. S. Gutteridge, and J. R. Norris, J. Gen. Microbiol. **104,** 67 (1978).
51. W. Windig, J. Haverkamp, and P. G. Kistemaker, Anal. Chem. **55,** 81 (1983).
52. R. Goodacre, M. J. Neal, and D. B. Kell, Zbl. Bakt. **284**, 516 (1996).
53. D. E. Rumelhart, J. L. McClelland, and the PDP Research Group, *Parallel Distributed Processing, Experiments in the Microstructure of Cognition* (MIT Press, Cambridge, Massachusetts, 1986), Vols. I and II.
54. P. D. Wasserman, *Neural Computing: Theory and Practice* (Van Nostrand Reinhold, New York, 1989).
55. P. J. Werbos, *The Roots of Back-Propagation: From Ordered Derivatives to Neural Networks and Political Forecasting* (John Wiley, Chichester, 1994).
56. S. S. Haykin, *Neural Networks: A Comprehensive Foundation* (Macmillan, New York, 1994).
57. R. Hecht-Nielsen, *Neurocomputing* (Addison-Wesley, Massachusetts, 1990).
58. D. M. Haaland and E. V. Thomas, Anal. Chem. **60,** 1193 (1988).
59. P. J. Brown, J. Chemometrics **6,** 151 (1992).
60. M. B. Seasholtz and B. Kowalski, Anal. Chim. Acta **277,** 165 (1993).
61. K. Joreskog and H. Wold, *Systems under Direct Observation* (North Holland, Amsterdam, 1982).
62. W. P. Carey, K. R. Beebe, E. Sanchez, P. Geladi, and B. Kowalski, Sens. Actuat. **9,** 223 (1986).
63. P. Geladi and B. Kowalski, Anal. Chim. Acta **185,** 1 (1986).
64. T. J. McAvoy, H. T. Su, N. S. Wang, M. He, J. Horvath, and H. Semerjian, Biotechnol. Bioeng. **40,** 53 (1992).
65. S. P. Jacobsson and A. Hagman, Anal. Chim. Acta **284,** 135 (1993).
66. P. Bhandare, Y. Mendelson, E. Stohr, and R. A. Peura, Vibr. Spectrosc. **6,** 363 (1994).
67. M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, and M. Redon, Anal. Chem. **67,** 4477 (1995).
68. J. T. Magee, in *Handbook of New Bacterial Systematics,* M. Goodfellow and A. G. O'Donnell, Eds. (Academic Press, London, 1993), p. 383.
69. W. H. Nelson and J. F. Sperry, in *Modern Techniques for Rapid Microbiological Analysis,* W. H. Nelson, Ed. (VCH Publishers, New York, 1991), p. 97.
70. W. H. Nelson, R. Manoharan, and J. F. Sperry, Appl. Spectrosc. Rev. **27,** 67 (1992).
71. D. Naumann, D. Helm, and H. Labischinski, Nature **351,** 81 (1991).
72. D. Naumann, D. Helm, H. Labischinski, and P. Giesbrecht, in *Modern Techniques for Rapid Microbiological Analysis,* W. H. Nelson, Ed. (VCH Publishers, New York, 1991), p. 43.
73. Y. W. Lai, K. Kemsley, and R. H. Wilson, J. Agric. Food Chem. **42,** 1154 (1994).
74. M. Defernez, E. K. Kemsley, and R. H. Wilson, J. Agric. Food Chem. **43,** 109 (1995).
75. Y. Ozaki, R. Cho, K. Ikegaya, S. Muraishi, and K. Kawauchi, Appl. Spectrosc. **46,** 1503 (1992).