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Determination of the adulteration of butter fat by its triglyceride composition obtained by GC. A comparison of the suitability of PLS and neural networks

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The suitability of partial least squares (PLS) and neural nets for the identification of mixtures of butter fat with foreign fat is compared. While neural nets are most suitable for classification, quantitative results are obtained by PLS. Butter fats of various European countries have been analyzed by GC. Fifty-six samples were used as the calibration set to build the PLS model and prepare the neural net, respectiveley. For successful modelling a 11-factor PLS model was sufficient. The neural net architecture chosen is a 17×1 preceptron. Both data evaluation techniques have been validated with 28 samples not included in the calibration set. For PLS the results indicate a detection limit of 1–2% foreign fat in butter fat. The neural net classified 20 samples correctly, but eight samples could not be classified at all. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

Milk fat because of its taste is regarded by the consumer as superior to other fats. Therefore its adulteration has always been a serious problem because of the economic advantages taken by partly replacing the high-priced milk fat with low-priced oils (e.g. sunflower oil) without labelling the product accordingly. Of all milk fat products butter is the most important one for economic reasons. The composition of butter fat is influenced by genetic factors (Gibson, 1991) and feeding conditions (Guyot, 1977*a,b*). Because of their overwhelming variety, the characterization of those natural samples is a very difficult task. Based on their fatty acid composition, the possible number of triglycerides in milk fat is calculated to be greater than 1300 (Barron *et al.*, 1990). Recently a review of the most commonly used methods for the detection of the triglyceride composition of butter fat has been compiled by the author (Lipp, 1995*b*). Generally it is considered as not possible to identify all triglycerides with only one chromatographic method (Kuksis *et al.*, 1991). The signals of the individual triglycerides show severe overlapping, therefore only multivariate methods are considered to be able to identify the amount of foreign fat added.

For this work gas chromatographic separation of triglycerides was used. This means that the triglycerides are separated according to their acyl-C numbers. They are separated into groups of identical numbers of acyl-C atoms. Based on these signals the data evaluation using partial least squares (PLS) regression or neural nets should allow the identification of mixtures of butter fat with foreign fat. As shown previously (Lipp, 1995*a*) the results of PLS correlate very well with the results of the method developed by Precht (1992; Molkentin & Precht, 1994). The latter is based on multiple linear regression and subject to an EU proposal becoming an officially recommended method of the EU.

PARTIAL LEAST SQUARES REGRESSION

Partial least squares (PLS) is a well known method for multivariate statistical data evaluation. It models a relation between the variables measured and a target variable, in this case the concentration of foreign fat, using a set of samples where the composition is known, the calibration set. The model obtained can then be used for the prediction of unknown samples. The theory of

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the method as well as some applications in food science has been described in a number of publications (e.g. de Jong, 1991; Gemperline, 1989; Haaland & Thomas, 1988; Kaufmann, 1993; Kvalheim, 1988; Thomas & Haaland, 1990; Wold, 1987).

PLS is closely related to the principal component analysis (PCA), and consists of matrix decomposition into a matrix of eigenvectors and a matrix of its loadings. Having a data matrix $\mathbf{X}_{N\times M}$ with N rows and M columns, the general approach can be written as

$$\mathbf{X}_{N\times M} = \mathbf{T}_{N\times A}\mathbf{P'}_{A\times M} + \mathbf{E}_{N\times M}.$$

This is equivalent to a reduction of the M-dimensional variable space to A-dimensional space. The dimensions A are also called latent variables. The matrix **T** contains orthogonal column vectors, also called score vectors, representing the latent variables. The rows of the matrix **P'** are the loadings of this latent variables and can be regarded as the covariances or correlations between the measured variable and a latent variable. The matrix **E** contains the residuals, that is all the variance in **X** not explained by the eigenvectors. PLS uses an iterative approach for the determination of as much variance as possible in the target variable by each factor computed. The spectral residuals, that is, the amount of spectral variance not explained by the model are used to characterize the quality of the model. All specimens should show about the same small amount of spectral residuals.

For PLS it is necessary to determine the number of factors which fit the model best. Too few factors would lead to higher imprecision because not all information both needed and available is used. Too many factors would also cause a decrease in the precision in prediction because too much 'noise', information not relevant for the model under investigation, is used. For this reason a predictive error of the sum of squares (PRESS) is calculated. Hereby starting with only one factor, the equation showing above is solved leaving one sample out. The target value of this sample is than calculated and compared to the real target value. The difference is squared and added to PRESS. This procedure is continued until each sample is left out once. Then the same procedure is performed using two factors, three factors and so on until a previously set maximum is reached. Two approaches exist for the determination of the number of significant factors. One approach sets the number of significant factors to the number of factors for which a minimum in PRESS is observed. The other procedure computes for every factor an F-test as an estimator of the significance of the decrease in PRESS and takes this number of factors for which the last significant F-test could be computed.

NEURAL NETWORKS

As network topology a feed forward net was chosen. This net generally is composed of an input layer, hidden layers and an output layer. The hidden layers are not mandatory, the other layers, of course, are. Each neuron is connected with all neurons of the next layer, except for the output layer. Different functions for the evaluation of the activation and the output of the neuron are available. The following description and nomenclature is taken from SNNS (1994). For this work the logistic function

$$a_j(t+1) = \frac{1}{1 + e^{-\Sigma_i \omega_{ij} o_i(t) - \theta_j}}$$

was used as an activation function for the neurons, where: $a_j(t)$ activation of unit j in step t; $o_i(t)$ output of unit j in step t; j index for some unit in the net; i index of a predecessor of the unit j; ω_{ij} weight of the link from unit i to unit j; Θ_j threshold (bias) of unit j.

The output function is the identity function $o_j(t) = a_j(t)$. As shown in the Results section this simple topology of a feed forward net was sufficient for the discrimination problem. It means that the 17 input neurons are directly linked to an output neuron, without any hidden layers. To compute the best weights for the links the so-called vanilla-backpropagation was used. The weights are updated for each learning cycle by

$$\delta\omega_{ij} = \eta\delta_j o_i \text{ with } \delta_j = t_j - o_j,$$

where: η learning factor (a constant); δ_j error (difference between the real output and the teaching output) of unit j; t_j teaching input of unit j; o_i output of the unit i; i index of a predecessor of at the current unit j with link ω_{ij} from i to j; j index of the current unit j.

Preparation of the network is performed by exposing each sample of the calibration set to the network and computing the weights accordingly. One cycle, where each sample is exposed exactly once to the network, is also called an epoch. To improve the understanding the sequence of the samples was randomly chosen.

Table 1. Experimental conditions

Gas chromatograph	GC 8700 (Perkin-Elmer, Milan)
Column	Packed column, 3% OV-1, 30 cm, diameter 2 mm (Chrompack, Milan)
Injector	Packed column injector at 370°C, injection volume 1 μl
Detector	Flame ionization detector at 370°C
Temperature program	1 min at 210°C to, at a rate of 6°C/min, 350°C for 12.7 min
Gas flow	He, 47 ml/min
Data acquisition	TurboChrom Ver 3.3 (Perkin-Elmer, Milan)

EXPERIMENTAL

The determination of the triglyceride composition was performed according to the conditions given in Table 1.

All sample ingredients were bought in supermarkets of the respective countries. The following fats, called foreign fats, have been used in preparing the mixtures: sunflower oil, soya oil, maize oil and lard. For the injection an aliquot of the samples has been diluted with *n*-heptan p.a. (Sigma, Milan) to form a 5% solution. In total 56 pure butter samples (19 from Germany, four from Belgium, 15 from Italy, 10 from France, five from Switzerland and two from Denmark) have been analysed. Four of the samples from Germany were labelled as light-products and had reduced fat and/or cholesterol content. The BCR RM 519 butter fat was also used for analysis. From these butter samples seven were chosen at random to form mixtures with sunflower oil, six to form mixtures with soya oil, five to form mixture with maize oil and 15 to form mixtures with lard.

As input data for all data evaluation the weight% values for the corresponding triglyceride were computed. For this the response factors for the individual groups of triglycerides were determined using preliminary results from the certification of the BCR reference butter fat BCR RM 519, in which the author's laboratory took part. All response factors were found to be close to 1. An excellent separation of all even numbered triacylglycerols ($2n$) starting at C_{24} was achieved. Figure 1 shows a typical chromatogram of a pure butter sample and Fig. 2 shows a chromatogram

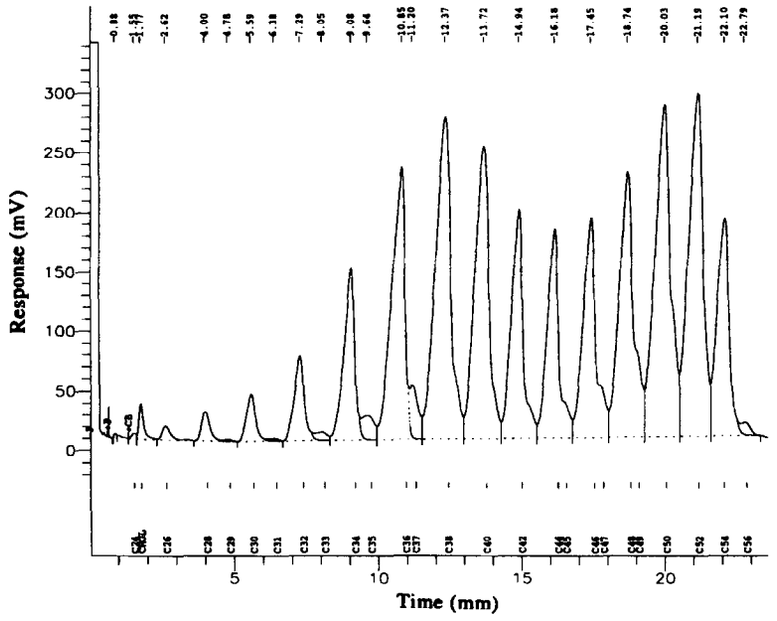

Fig. 1. Chromatogram of a pure Swiss butter sample (No. 56 of the calibration set).

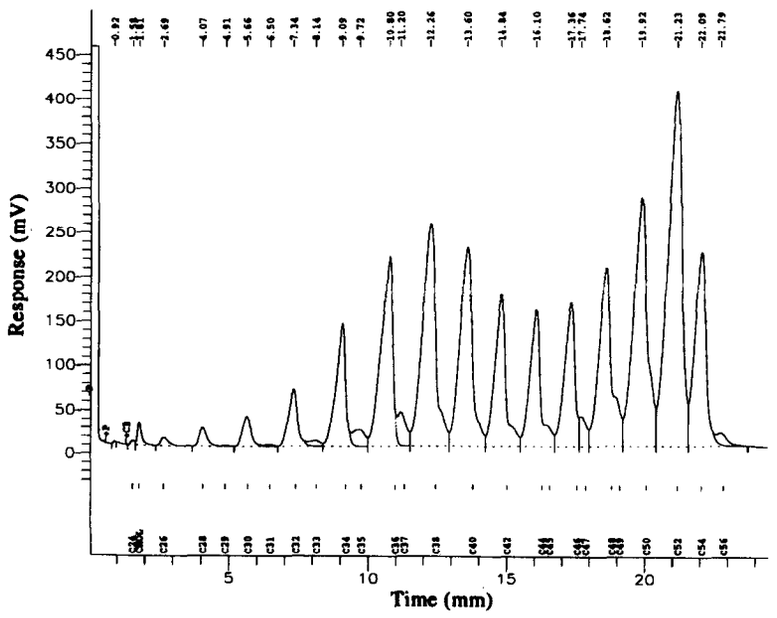

Fig. 2. Chromtogram of a mixture of the butter shown in Fig. 1 containing 11.7% lard (No. 7 of the calibration set).

Table 2. Composition of mixtures used in calibration and prediction set

Code	Description	Code	Description
Calibration set			
2	Italian butter with 5.7% lard	6	Italian butter with 5.4% lard
7	German butter with 11.7% lard	11	Italian butter with 4.3% lard
13	Italian butter with 2.9% lard	16	German butter with 1.8% maize oil
18	Belgian butter with 4.3% sunflower oil	20	Belgian butter with 4.6% sunflower oil
22	Belgian butter with 2.3% soya oil	24	German butter with 7.4% soya oil
26	Irish butter with 6.9% soya oil	31	German butter with 10.4% maize oil
33	German butter with 8.4% soya oil	36	German butter with 9.9% lard
39	Italian butter with 10.2% maize oil	40	Italian butter with 12.3% lard
43	Italian butter with 10.5% sunflower oil	44	Italian butter with 9.5% soya oil
47	Italian butter with 3.9% soya oil	50	Danish butter with 6.1% sunflower oil
51	Swiss butter with 8.4% sunflower oil	54	Italian butter with 5.4% lard
55	Italian butter with 13.4% lard		
Prediction set			
2	French butter with 8.2% lard	4	Italian butter with 7.7% lard
5	Italian butter with 5.4% lard	6	BCR fat with 6.2% maize oil
8	Italian butter with 2.1% lard	9	German butter with 9.2% sunflower oil
13	German butter with 10.7% sunflower oil	16	Swiss butter with 8.1% maize oil

for a mixture of this butter with the addition of 11.7% (w/w) lard. The odd numbered $(2n+1)$ triacylglycerols could not be separated in all cases, therefore their area is added to the prior eluted even numbered $(2n)$ peak. The low content of C_{56}, being less reproducible, was ignored.

The multivariate statistical data analysis was performed using the PLSplus package Ver.2.1G for GRAMS/386 (Galactic, Salem, USA). The software was run on a NEC Powermate 386/33i, supplied with a mathematical Coprocessor.

All simulations of the neural networks have been computed using SNNS Ver. 3.3 (Stuttgart Neural Network Simulator). This versatile tool is available as free software via ftp.* The simulator is available for various platforms and has been used on an IBM RS 6000/355 workstation under AIX V3.2.

The data are divided into two sets, with a calibration set of 56 samples and a prediction set of 28 samples (see Table 2). The first data set was used to calibrate the PLS model and to prepare the neural net, while the second set was used for prediction. The amount of foreign fat added was known and used to show the quality of the prediction properties of the two methods. All data were subject to mean centering and scaling variance prior to use in the respective models. Details of this data pretreatment can be found in, for example, Kowalski (1984) and Martens & Næs (1989).

RESULTS

Partial least squares, PLS

The PLS analysis of 56 butter fat samples and mixtures leads to a calibration model with 11 significant factors. The standard error for prediction, defined as the root mean square of the difference between predicted and actual concentrations of this model, is computed to be 0.42. Figure 3 shows a graph of the actual vs predicted concentration of foreign fat in butter fat for the samples used in setting up the PLS model. This graph indicates that the detection of the addition of at least 2% of foreign fat is possible using PLS for data evaluation.

Figure 4 shows in more detail the prediction errors for each sample used in the calibration model. There is no sample for which the error of prediction exceeds 1% significantly. The standard deviation of the residuals of concentration is calculated to be 0.34. In general the detection limit is regarded to be 3 times this standard deviation. This also encourages the statement that the detection limit should not exceed 2% for the detection of foreign fat within European butter samples. To show in more detail the quality of the PLS model the spectral residuals, that is the amount of spectral variance not explained by the PLS model, are shown in Fig. 5. As the

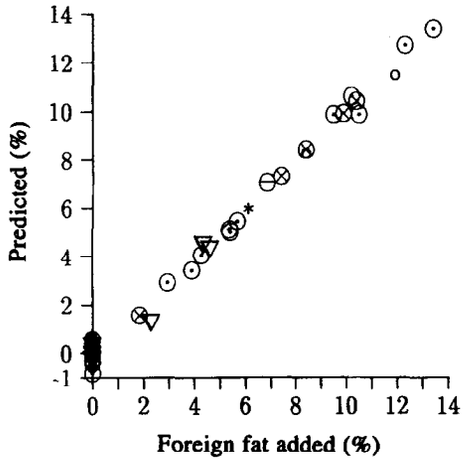

Fig. 3. Predicted vs actual foreign fat concentration, PLS calibration set. ⊗, German; ⊙, Italian; ○, Swiss; ◇, French; *, Danish; ⊖, Irish; ▽, Belgian; and ×, BCR butter fats.

*Via anonymous ftp from host: ftp.informatik.uni-stuttgart.de

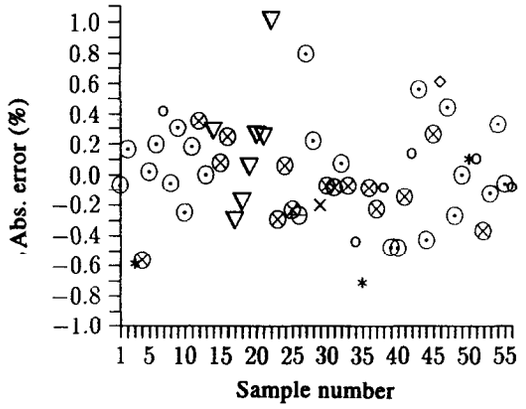

Fig. 4. Residuals of concentration of the PLS calibration set. ⊗, German; ⊙, Italian; ○, Swiss; ◇, French; *, Danish; ⊖, Irish; ▽, Belgian; and ×, BCR butter fats.

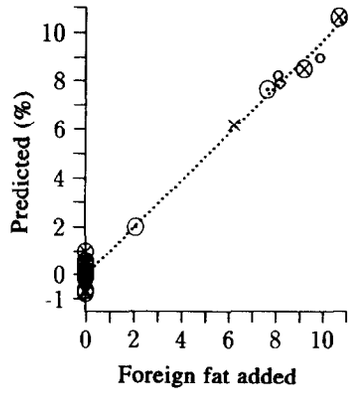

Fig. 6. Prediction of foreign fat contents of 28 fat samples by PLS. ⊗, German; ⊙, Italian; ○, Swiss; ◇, French; and ×, BCR butter fats; results of the least square regression on the data are drawn as a dotted line, the regression coefficient is computed to be $r^2 = 0.988$.

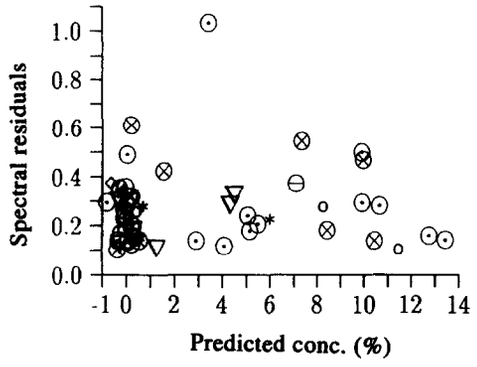

Fig. 5. Spectral residuals of the PLS calibration set. ⊗, German; ⊙, Italian; ○, Swiss; ◇, French; *, Danish; ⊖, Irish; ▽, Belgian; and ×, BCR butter fats.

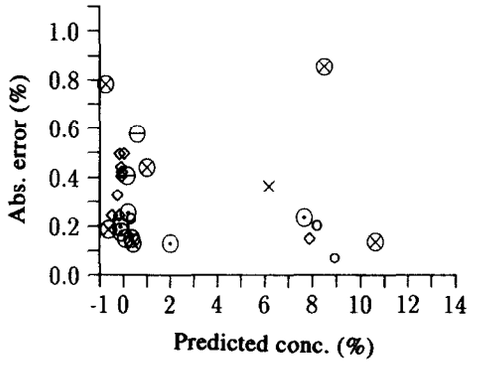

Fig. 7. Spectral residuals of the PLS prediction set. ⊗, German; ⊙, Italian; ○, Swiss; ◇, French; *, Danish; ⊖, Irish; ▽, Belgian; and ×, BCR butter fats.

input data are weight%, the spectral variance totals 100%. For this reason the spectral residuals equals the amount of weight% which are not modelled by the 11-factor model of PLS. As there are only small residuals, generally under 1%, observed the PLS model fits the calibration data very well. As there was no trend regarding the concentration of foreign fat, the spectral residuals seems not to be effected by the addition of foreign fat. This indicates that the present data show a strict linearity emphasizing that the application of PLS was appropriate.

To test this calibration model 28 pure butter fats and mixtures have been analyzed and their concentration of foreign fat was predicted using the PLS model. These samples of course have not been included in the calibration set. Figure 6 shows the predicted amount of foreign fat vs the real amount of foreign fat added. The deviation is less than 1% and only slightly exceeds 1% for one specimen. In order to evaluate the calibration samples further the spectral residuals of these data are shown in Fig. 7. All residuals are very small, thus indicating that the calibration set was representative for all samples under investigation. PLS seems to be appropriate for extracting the relevant data for the prediction of foreign fat in butter fat. The data indicate again that the PLS model fits well and allows an easy and fast way for the determination of foreign fat in European butter samples if the amount of foreign fat added exceeds 1%.

Neural networks

The same data sets as for PLS has been investigated by a feed forward neural net. Seventeen input neurons were connected to one output neuron, thus forming a 17×1 perceptron. These architecture is suitable for classification only, therefore it was only tried to differentiate between pure butter fat and mixtures of butter fat and foreign fat. As the same data are used as for PLS, it is obvious that the minimum concentration of foreign fat in butter fat was about 2%. So the net was prepared with only mixtures of more than 2% foreign fat in butter. All data have been recoded to indicate only the presence of foreign fat added. Mixtures of butter fat and foreign fat were coded as '1' while pure butter fat was coded as '0'. These modified data of the 56 specimens of the calibration set have been used to prepare the network. To prevent overfitting of the data, all values of the output neuron between 0 and 0.2 were regarded as '0' and all values between 0.8 and 1 as '1'. The learning parameter η, see above, was adjusted to 1. Only less than 30 epochs were needed to prepare the network

Table 3. Weights of the links from the input neurons to the output neurons in the 17×1 feed forward neural net for the determination of foreign fat in European butter fat

Neuron	Peak	Weight
1	C_{24}	0.711
2	Cholesterol	−0.405
3	C_{26}	0.068
4	C_{28}	−0.415
5	C_{30}	0.658
6	C_{32}	0.965
7	C_{34}	−0.427
8	C_{36}	−0.75
9	C_{38}	−0.689
10	C_{40}	−2.291
11	C_{42}	−1.591
12	C_{44}	−0.037
13	C_{46}	−1.497
14	C_{48}	−2.187
15	C_{50}	−1.781
16	C_{52}	−0.427
17	C_{54}	0.548

properly. The weights of the links between input and output neurons are shown in Table 3. The 28 samples of the prediction set were exposed to the prepared net. They were all coded in the same way as described above. From this set 20 samples were correctly identified. However the remaining eight samples were not classified at all using the net. The data are summarized in Fig. 8. Their predicted values lie well within the 0.2–0.8 range. As classification is only obtained between 0 and 0.2, and 0.8 and 1, respectively, these eight values could not be included in any classes. Within those eight samples there were seven pure fats and one mixture. It is worth noting that no incorrect classification occurred.

DISCUSSION

Both PLS and neural nets seem to represent versatile tools for the identification of the presence of 2% and more of foreign fat in butter samples from several European countries. While neural nets are only used here for the classification of the samples in pure butter fats and mixtures, PLS also generates quantitative results. The results indicate that PLS extracts the spectral information, which corresponds to the amount of foreign fat, more easily than the feed forward neural net. While correct prediction of the 28 samples of the prediction set caused no problems to PLS, the neural net succeeded in characterizing 20 out of 28. However the remaining eight samples were not classified at all and are easily recognizable as outliers regarding the current training of the neural net. Preparation of the net with all data indicated that the 17×1 design of the neural net should be appropriate to identify the presence of foreign fat in butter if the calibration set consisted of representative samples. PLS extracts enough information from the 56 calibration samples to correctly predict the 28 samples of the prediction set. So PLS

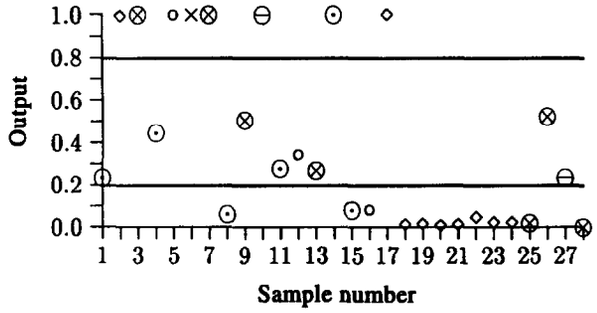

Fig. 8. Results of the prediction of 28 samples by the 17x1 perceptron. Pure butter fat coded as '0', mixtures as '1'. Values between 0 and 0.2 are regarded as 0, while values between 0.8 and 1 are regarded as 1. ⊗, German; ⊙, Italian; ○, Swiss; ◇, French; ⊖, Irish; and ×, BCR butter fats.

seems more able than neural nets to extract the significant patterns in small calibration sets.

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REFERENCES

Barron, L. J. R., Hierro, M. T. G. & Santa-Maria, G. (1990). HPLC and GLC analysis of triglyceride composition of bovine, ovine and caprine milk fat. *J. Dairy Res.*, **57**, 517.

de Jong, S. (1991). Chemometrical applications in an industrial food research laboratory. *Mikrochim. Acta*, **2**, 93.

Gemperline, P. (1989). Mixture analysis using factor analysis. I: calibration and quantitation. *J. Chemom.*, **3**, 549–68.

Gibson, J. P. (1991). The potential for genetic change in milk fat composition. *J. Dairy Sci.*, **74**, 3528.

Guyot, A. (1977*a*). Chromatographie gaz–liquide des triglycérides de la matière grasse butyrique I. Variations de la composition en triglycérides de la matière grasse des beurres. *Bull. Rech. Agron. Gembloux*, **12**, (4), 299.

Guyot, A. L. (1977*b*). Chromatographie gaz–liquide des triglycérides de la matière grasse butyrique. II. Applications à, quelques problèmes de controle. *Bull. Rech. Agron. Gembloux*, **12**, (4), 311.

Haaland, D. M. & Thomas, E. V. (1988). Partial least square methods for spectral analysis. *Analyt. Chem.*, **60**, 1193.

Kaufmann, P. (1993). Prediction of mixture composition by chromatographic characterization, multivariate classification and partial least-square regression, a comparison of methods. *Anal. Chim. Acta*, **277**, 467.

Kowalski, B. R. (1984). *Chemometrics*, Vol. 138. *NATO Advanced Science Institute Series C*. Reidel, Dordrecht.

Kuksis, A., Marai, L., Myher, J. J., Itabashi, Y. & Pind, S. (1991). Qualitative and quantitative analysis of molecular species of glycerolipids by HPLC. In *Analysis of Fats, Oils and Lipoproteins*, ed. E. G. Perkin. American Oil Chemists' Society.

Kvalheim, O. M. (1988). Interprtation of direct latent-variable projection methods and their aims and use in the analysis of multicomponent spectroscopic and chromatographic data. *Chemom. Intell. Lab. Syst.*, **4**, 11.

Lipp, M. (1995*a*). Comparison of PLS, PCR, and MLR for the quantitative determination of foreign oils and fats in butter fat of several european countries by its triglyceride composition. *Z. Lebensm. Unters. Forsch.*, in press.

Lipp, M. (1995*b*). Review of methods for the analysis of triglycerides in milk fat: application for studies of milk quality and adulteration. *Food Chem.*, **54,** 213–21.

Martens, H. & Næs, T. (1989). *Multivariate Calibration.* John Wiley and Sons, Chichester.

Molkentin, J. & Precht, D. (1994). Comparison of packed and capillary columns for quantitative gas chromatography of triglycerides in inilk fat. *Chromatographia*, **39,** (5/6), 26.

Precht, D. (1992). Bestimmung der Reinheit von Milchfetten mit gaschromatographischen Methoden—Eine aktuene Aufgabe einer EG-Expertengruppe. *Dmz Lebensmittelind. Milchwirt.*, **27,** 796.

SNNS (1994). *SNNS Stuttgart Neural Network Simulator, User Manual, Version 3.3.1.* Institute for Parallel and Distributed High Performance Systems, University of Stuttgart.

Thomas, E. V. & Haaland, D. M. (1990). Comparison of multivariation calibration methods for quantitative spectral analysis. *Analyt. Chem.*, **62,** 1091.

Wold, S. (1987). Principle component analysis. *Chemom. Intell. Lab. Sys.*, **2,** 37.