ELSEVIER

Chemometrics and Intelligent Laboratory Systems 34 (1996) 85–102

Chemometrics and intelligent laboratory systems

Enzymatic browning of vegetables. Calibration and analysis of variance by multiway methods

Rasmus Bro [a,*], Hanne Heimdal [b]

[a] *Chemometrics Group, Food Technology, Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark*

[b] *Department of Dairy and Food Science, Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark*

Received 15 November 1995; accepted 25 March 1996

Abstract

This paper describes the chemometrical aspects of an investigation of the enzymatic browning of vegetables. Enzymatic browning is caused by polyphenol oxidase, PPO. Kinetic UV/VIS spectra and experimental design variables of PPO incubated samples are used for predicting enzymatic activity and substrate consumption. The mathematical models used are multiway PLS (N-PLS) and fiveway PARAFAC. Both methods are available from Internet in MATLAB code. Throughout the results of the multiway methods are compared to competing methods (PLS, PCR, Tucker, feedforward neural networks, locally weighted regression, ANOVA and others). The result of the investigation is, that the multiway methods have clear advantages with respect to predictions and interpretability, both mathematically and technologically.

Keywords: Enzymatic browning of vegetables; Muliway methods; Polyphenol oxidase

1. Introduction

Undesirable browning effects in fruit and vegetables have been investigated in several surveys, yet little is still known on the basic mechanisms controlling the browning. A major contributor to browning is the enzymatic browning caused by PPO, polyphenol oxidase [1].

Enzymatic browning is sometimes expressed as the dioxygen consumption of PPO, which is directly related to the activity of PPO, and sometimes as formation of color. One purpose of this investigation is to verify if these two ways of expressing the impact of PPO are interchangeable in some way and therefore, if both are valid to draw conclusions from. The relation between dioxygen consumption, color formation and substrate degradation is modelled, and the relation between PPO activity and O_2, CO_2, pH, substrate and temperature is determined by response surface design.

The main part of the paper is divided into three parts describing different modelling problems of a model system of lettuce:

– Predicting PPO activity (dioxygen consumption) from kinetic UV/VIS spectra. Fortyfive samples with different substrates were incubated with

* Corresponding author. E-mail: rasmus.bro@pop.foodsci.kvl.dk.

PII S0169-7439(96)00019-6

PPO. The activity of PPO was determined by PPO assay and the UV/VIS spectra were measured several times during an hour from incubation. The relation between the kinetic spectra and the activity was found by multiway PLS (N-PLS) with a preprocessing step designed to eliminate the nonlinearities in the data. The purpose of the model is to show, that the spectral changes in the model system are indeed indicative of the PPO activity and to show how the spectral changes relate to the activity.

– Predicting kinetic changes in the amount of products (determined by HPLC) from kinetic UV/VIS spectra. The model is essentially the same as before, albeit now the dependent variable is multivariate, namely the substrate consumption determined by HPLC after five different incubation times. Predicting the outcome of the HPLC analysis from spectra would make it possible to avoid the tedious and chemistry consuming HPLC analysis.

– Describing the influence of five different factors on the PPO activity (dioxygen consumption). The data in this problem can be regarded as a fiveway array of activities and decomposing this array to its essentials by PARAFAC can give a model that simultaneously makes it possible to predict the activity of other levels of factors than the ones used and show how the factors influence the PPO activity.

Each part consists of a description of the mathematical model used, an essential part describing the results and how they are obtained, and finally a description of the results of using other methods is given. By N-PLS and PARAFAC it is demonstrated how the use of suitable mathematical methods coupled with the right preprocessing can help understanding underlying bio-physical phenomena in complicated systems.

For details on the experimental conditions and a more in-depth discussion of the technological aspects see Ref. [2].

2. Materials and methods

2.1. General

Five heads of fresh iceberg lettuce (Lactuca sativa L. cv. Saladin 'Iceball') were obtained from a commercial grower near Copenhagen, Denmark. PPO was extracted and purified according to Ref. [2].

Table 1
Experimental design for the three different experiments

Factor	Levels (UV/VIS)	Levels (HPLC)	Levels (PPO activity)
Substrate	CG, EPI, MIX	CG, EPI, MIX	CG, EPI, MIX
O_2 (%)	0, 5, 10, 20, 80	0, 20, 80	0, 5, 10, 20, 80
CO_2 (%)	0, 10, 20	0, 20	0, 10, 20
pH	6	6	3.0, 4.5, 6.0
Temp. (°C)	30	30	5, 20, 30
# samples	45	15	405

All combinations performed except for $O_2/CO_2 = 0/20$ in the HPLC analysis. In the UV/VIS experiment UV/VIS spectra were measured. In the HPLC experiment the area of the substrate peak was determined relative to an internal standard, and in the PPO activity experiment, the PPO activity (dioxygen consumption), was determined in replicates.

Color was measured spectrophotometrically with a Lambda 19 UV/VIS/NIR spectrometer (Perkin Elmer) from 220 to 500 nm (1 nm intervals) every second minute 30 times. Measurements were made in a closed quartz cuvette containing 3.555 ml 0.1 M acetate buffer (pH 6.0, 30°C) saturated with a gas mixture of appropriate composition, 25 μL of either 0.01 M chlorogenic acid (CG), 0.01 M epicatechin (EPI) or an equimolar mixture (0.01 M) of both (MIX), and 20 μL purified PPO (Table 1). Almost only the substrate (CG/EPI/MIX) and its products absorb light in the samples, and the changes in absorbance therefore relate directly to the changes in the substrate.

Substrate consumption was determined by reverse phase HPLC using a series 1050 HPLC (Hewlett Packard), ODS Hypersil C18 column. The detection method was UV absorbance at 280 nm and a gradient method according to Ref. [3] was used. Solvent A was water at pH 3.0 adjusted with 1 M phosphoric acid and solvent B acetonitrile. The amount of each substrate is expressed as peak area relative to the peak area of the internal standard (0.2 mM vanillic acid). The sample contained 4.775 ml 0.001 M acetate buffer (pH 6.0, 30°C) saturated with a gas mixture of appropriate composition, 0.1 mL 0.04 M chlorogenic acid, epicatechin or a mixture of those, and 25 μL purified PPO (Table 1). The sample reacted in a Clark electrode chamber aerated with the same gas mixture

(Rank Brothers, UK) and for each time (0, 1, 5, 10, and 30 min) 0.2 mL was sampled from the chamber and immediately mixed with an equal volume of stopping solution containing 2 mM NaF, 2 mM cysteine and 0.2 mM vanillic acid. The samples were stored at −5°C until analysis (the same day).

PPO activity was measured in nanomoles of O_2 consumed per second by a polarographic polyphenol oxidase assay as described in Ref. [4]. PPO activity was determined for various levels of five different factors: O_2, CO_2, temperature, pH, and substrate type (Table 1).

2.2. Predicting enzymatic activity from UV / VIS spectra

UV/VIS spectra were measured at 30 equidistant times during one hour from incubation with PPO on samples with three different substrate types, five different O_2 levels and three different CO_2 levels. All factors varied independently giving a total of 45 samples. The corresponding activity of PPO was determined by a polarographic polyphenol oxidase assay. With these data it is possible to investigate the appearance of color at various conditions.

One UV/VIS spectrum consists of 281 wavelengths. The matrix of all measured spectra for one sample is a 281×30 matrix, and the array of independent variables for all samples is thus $45 \times 281 \times 30$. The dependent variable is a 45×1 vector of activities.

2.3. Predicting chromatographic peaks from kinetic UV / VIS spectra

The development of color in the simple model system can be expressed as the formation of products. By HPLC the consumption of substrate was measured at some of the same conditions as for the spectral investigation. The consumption of substrate is directly related to the amount of products. Predicting the kinetic profile of substrate from the former determined spectra, thus implies that the influence of PPO can be determined not only as the dioxygen consumption of PPO, but also as the amount of transformed substrate. The substrate consumption was measured at five different times after incubation with PPO (0, 1, 5, 10 and 30 min). The dependent variable is therefore five-dimensional and is arranged in a 15×5 matrix corresponding to the 15 samples determined.

2.4. Predicting PPO activity from experimental design

For three substrate types, five O_2 levels, three CO_2 levels, three pH values and three different temperatures — all varied independently — the activity of PPO was determined in replicate. Building a calibration model to predict the activity from the experimental conditions would give important information on how the PPO activity — and therefore the color formation — is influenced by the different factors. The different levels of the factors are shown in Table 1. The number of samples in the replicated full factorial design is $3 \times 5 \times 3 \times 3 \times 3 \times 2 = 810$. Most of the time the mean value of the replicates will be used, giving a total of 405 samples. How the data was structured depends on the analysis and will be described later.

To clarify the experiments Table 1 shows the factors varied in the three performed experiments. All calculations were done on a 133 MHz Dell PC with 32 Mb RAM. The trilinear PLS algorithm was made in the mathematical software Matlab for Windows 4.2c.1 (Mathworks, Inc.). This implementation works with arrays of independent variables up to fiveway and arrays of dependent variables up to threeway. The PARAFAC algorithm was made in Matlab and works with up to tenway arrays. It also contains the possibility to constrain loadings to be orthogonal or non-negative and handles missing data. The algorithms are available from Internet at http:\\newton.foodsci.kvl.dk\foodtech.html. Also available from our homepage are M-files for PARAFAC and Tucker made by Claus A. Andersson, from where the Tucker algorithm was obtained. The LWR algorithm used was taken from the chemometrical toolbox made by Wise and Gallagher [5] which runs under MATLAB. The neural network is part of the Neural Network Toolbox from Mathworks, Inc. ANOVA was performed in SAS. All other algorithms were implemented in-house in Matlab.

3. Predicting enzymatic activity from UV / VIS spectra

3.1. Mathematical model

For a set of calibration samples a cube or threeway array (sample × spectra × time) of independent variables is obtained as shown in Fig. 1.

A calibration model is sought to predict the activity from the kinetic UV/VIS data in the threeway cube. The mathematical model is an extension of the PLS algorithm [6] to higher orders called multiway PLS or N-PLS [7], specifically trilinear PLS. Trilinear PLS is suited for the datatype dealt with here. Each latent variable in the trilinear model consists of a loading vector in the spectral direction, a loading vector in the kinetic direction and a score vector in the sample direction. The latent variables are found successively to have maximal covariance with the yet unexplained variation in the activity. The advantages of using a multiway method compared to ordinary twoway multivariate methods have been described by several authors (e.g. Ref. [8]). N-PLS is superior to unfolding methods, primarily due to a stabilization of the decomposition, because fewer parameters need to be estimated. Therefore more degrees of freedoms can be used in estimating the parameters of the model, giving a more robust, parsimonious and interpretable model.

The trilinear PLS algorithm will here be described mathematically and to some extent qualitatively. For a more thorough description see Ref. [7]. In the following, scalars are shown as lower-case italics, vectors by bold lower-case characters, bold capitals are used for twoway matrices and underlined bold capitals for threeway arrays. The letters I, J, K and M are reserved for indicating the dimension of different orders. The terms mode, way and order are used more or less interchangeably though a distinction should be made between the geometrical dimension of the hypercube — the number of ways — and the number of independent ways — which is the order/mode [9] [10].

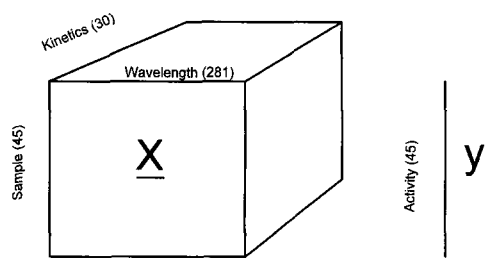

Fig. 1. The structure of the calibration data. For each sample a matrix of spectra at different times is measured.

The independent variables are arranged in a threeway cube called $\underline{\mathbf{X}}$ ($I \times J \times K$). In this case I is the number of samples, J the number of wavelengths measured and K the number of times the spectrum of each sample is measured. The dependent variable is arranged in an $I \times 1$ vector $\mathbf{y}$ or an $I \times M$ matrix $\mathbf{Y}$ in case of several dependent variables. The original algorithm also handles higher order $\underline{\mathbf{X}}$ and $\mathbf{y}$, but only the relevant algorithm will be described here.

Both $\underline{\mathbf{X}}$ and $\mathbf{y}$ are centered by subtracting the mean of each observation over all samples. Mathematically this can be described for $\underline{\mathbf{X}}$ as

$$x^{c}_{ijk} = x_{ijk} - \overline{x_{jk}}$$

In the formula x_{ijk} is the absorbance of the ith sample on the jth wavelength at the kth time. The subscript c indicates that it is the centered value. Mean-centering removes any offset in the data, and thus eliminates the need for any offsets in the following regression [11,12]. Furthermore it has the advantage that the decomposition is done with respect to the *variations* in the data instead of the level. Preprocessing of multiway data in general is more complicated, than preprocessing twoway data, because the preprocessing can have gross impact on the multilinear structure assumed to be in the data [13–15].

To calculate the first latent variable the product of the cube $\underline{\mathbf{X}}$ and the vector $\mathbf{y}$ is calculated as

$$z_{jk} = \sum_{i=1}^{I} x_{ijk} y_i .$$

The thereby formed $J \times K$ matrix called $\mathbf{Z}$ is decomposed by singular value decomposition [16]. The first set of normalized singular vectors can be shown to be the two weight vectors, that is sought for the first latent variable. The weight vectors are called $\mathbf{w}^J$ ($J \times 1$) and $\mathbf{w}^K$ ($K \times 1$), where the subscripts indicate that the vectors refer to the spectral and the kinetic order, respectively. With the weight vectors the

score vector can be computed, as the vector that produces the best least squares model of $\underline{\mathbf{X}}$ given the two weight vectors. The score vector, $\mathbf{t}$, is equal to

$$t_i = \sum_{j=1}^{J} \sum_{k=1}^{K} x_{ijk} w_j^J w_k^K,$$

t_i being the ith element of $\mathbf{t}$.

The latent variable defined by $\mathbf{t}$, $\mathbf{w}^J$ and $\mathbf{w}^K$ is the best least squares model of $\underline{\mathbf{X}}$, i.e.

$$\hat{x}_{ijk} = t_i w_j^J w_k^K, \quad (1)$$

under the constraint, that $\mathbf{t}$ has maximal covariance with $\mathbf{y}$. Thereby it is ensured not only to get a good model of $\underline{\mathbf{X}}$, but a good model, that is descriptive also of the variations in $\mathbf{y}$. $\hat{x}_{ijk}$ is the prediction of x_{ijk}.

The latent variable is related to $\mathbf{y}$ by ordinary linear regression giving a regression coefficient and a prediction of $\mathbf{y}$. The regression coefficient is calculated as

$$b = (t^{\mathrm{T}} t)^{-1} t^{\mathrm{T}} y$$

and the prediction of $\mathbf{y}$ is

$$\hat{\mathbf{y}} = \mathbf{t} b. \quad (2)$$

As can be seen, by calculating one latent variable, a model is obtained of both $\underline{\mathbf{X}}$ and $\mathbf{y}$, and both can be assessed in different ways to ensure, that they are valid [17]. In complex systems it is to be expected, that *one* latent variable is not enough to make a good model of $\mathbf{y}$. To calculate further latent variables the residuals (unexplained part) of both $\underline{\mathbf{X}}$ and $\mathbf{y}$ are calculated by subtracting the model so far from the actual data (the models given by Eqs. (1) and (2)). No extra loading vectors are calculated as in ordinary PLS, because this will not produce orthogonal scores anyway. In this respect the N-PLS algorithm resembles the PLS algorithm originally proposed by Martens and Næs [17].

The next latent variable is calculated by the same procedure as described above, but replacing $\underline{\mathbf{X}}$ and $\mathbf{y}$ with their residuals. When regressing the following score vectors on the residuals of $\mathbf{y}$ one has to incorporate earlier found score vectors as these are not orthogonal. This is done by arranging the score vectors in an $I \times f$ matrix, $\mathbf{T}$, where each column is a score vector. The regression coefficients for the fth latent variable are then found as

$$\mathbf{b} = (\mathbf{T}^{\mathrm{T}} \mathbf{T})^{-1} \mathbf{T}^{\mathrm{T}} \mathbf{y}_{\mathrm{res}},$$

where $\mathbf{y}_{\mathrm{res}}$ now means the unexplained part of $\mathbf{y}$.

It is worthwhile noticing, that the trilinear PLS corresponds to the Martens implementation of *bilinear* PLS with only one extra step included, if the threeway array is unfolded to a matrix. The ordinary weight vector found as $\mathbf{X}'\mathbf{u}$ (or $\mathbf{X}'\mathbf{y}$) is folded to a matrix with the dimension given by the number of variables in the second and third order of $\underline{\mathbf{X}}$ and this matrix is decomposed by SVD. The first pair of sin-

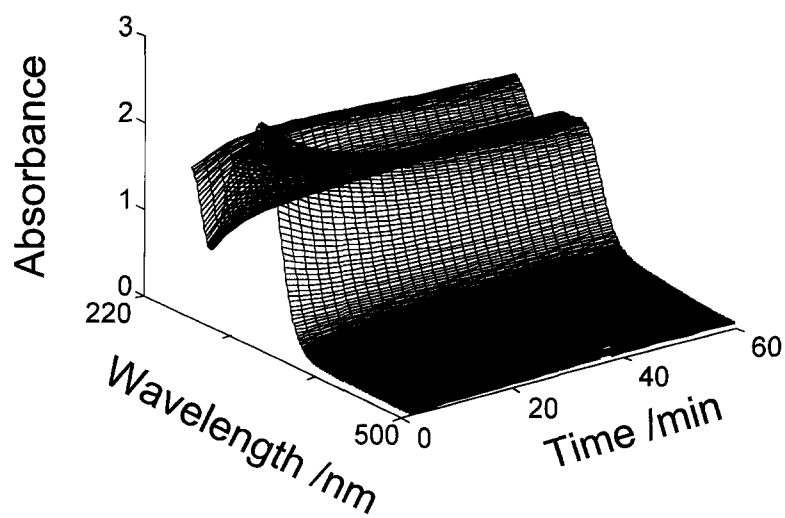

Fig. 2. A plot of a typical CG sample.

gular vectors is then the first set of weight vectors but can be backfolded to one ordinary weight vector by properly arranging the outer product of the two vectors, i.e. backfolding the matrix to a $JK \times 1$ vector. The rest of the algorithm will be exactly as the bilinear algorithm.

The algorithm described above is the threeway version of the so-called PLS1 algorithm. The number 1 indicate, that it deals with univariate **y**. If instead the dependent variables are twoway, they can be arranged in an $I \times M$ matrix, where M is the number of dependent variables. The PLS2 algorithm handles this situation automatically by decomposing **Y** in a manner similar to $\underline{\mathbf{X}}$. The implementation of the trilinear PLS2 can be seen in Ref. [7]. The advantage of calibrating for several variables simultaneously is especially pronounced when these are correlated. If spectra are to be predicted it is clearly better to predict the whole spectrum simultaneously, than predicting one wavelength at a time. Thereby the correlations between the dependent variables are used to stabilize the predictions.

3.2. Results and discussion

A visual inspection of the data immediately made it clear, that one experiment for each substrate-type failed ($O_2 = 80$, $CO_2 = 20$) in the UV/VIS experiment. The spectra were of much less magnitude, than expected from the measured activity and the biochemical knowledge. The reason is most likely, that the substrate was too old when this experiment was performed, and hence the substrate had decomposed non-enzymatically. As a consequence of that, these three experiments were deleted from the data giving a total of 42 samples. A typical sample is shown in Fig. 2.

The purpose of the calibration model is to incorporate the various levels of substrate, O_2 and CO_2 thereby showing that the effect of these factors with respect to activity can be determined from spectral measurements of the sample. Only few samples (42) were available, and it was very difficult to incorporate all the above-mentioned factors in one global model. Instead a local model was made for each of the three substrate types (EPI, CG, MIX). For each substrate there were then 14 samples.

The spectra were all measured from 220–500 nm, but for CG and MIX only 240–500 nm was used. The lower part was too noisy to be of any relevance. For epicatechin (EPI) the area 220–300 nm was furthermore deleted. The reason for this is both the fact that a model including this area is worse than a model without, and the fact that the main spectral variation for EPI products is in the VIS area. Furthermore the wavelengths 402–403 nm were removed due to an instrumental error in this wavelength area [2].

During analysis of the data it was clear that no linear relation could adequately describe the relation between the variations in the spectra and the variations in PPO activity. This is not surprising considering the complicated polymerization mechanisms and dependency of the activity of an enzyme on the concentration of substrate or other chemical conditions (cf. Michaelis–Menten). Another complicating problem is the special kind of closure in the data. All spectrally active analytes in the mixture stem from the substrate initially introduced, and there is therefore an interdependency between the analytes, which is defined by the polymerization and enzymatic kinetics. Finally there are differences in the initial amount of substrate from sample to sample. Because only few experiments could be performed on one day, a choice had to be made on whether to use the same substrate for all samples giving a sampling error from the instability of the substrate or use freshly made substrate every day giving a sampling error from the preparation of the substrate. It was chosen to use the same substrate for all samples, but the result was, that the substrate composition was not exactly the same in all experiments. The first measured spectrum for each sample, should be the same, if the substrate did not change, but a score plot of a PCA on the first set of spectra for e.g., CG clearly showed large systematic variations, that corresponded very well to the order in which the experiments were performed.

Summarizing, there are three things presumed to cause the nonlinearities: (i) The biological dependency of PPO activity on substrate, O_2 and CO_2, (ii) the closure, in that the weighted sum of spectral analytes is constant during an experiment, and (iii) the fact that the substrate composition is not the same from experiment to experiment. The last point in any case ensures that the experiments are more realistic (though this was not the intention!). It also makes it

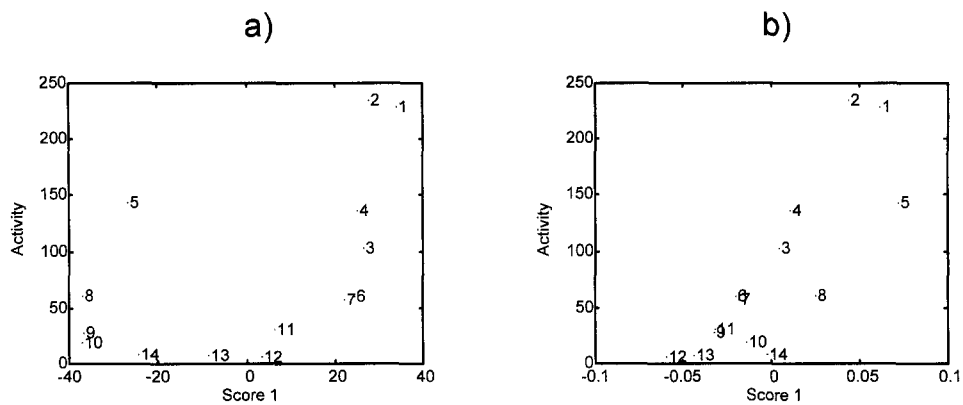

Fig. 3. Scatter plot from a model of CG data. (a) A plot of the activity against the first score vector from an N-PLS model without preprocessing, and (b) the same but with all sample matrices scaled no norm one.

necessary to incorporate the kinetics in the analysis. It is not possible to predict the activity from any level of a spectrum of the substrate or its products when the initial amount changes from experiment to experiment.

As an example of the nonlinearity in the data, the first score vector from an N-PLS model of CG data versus the activity is depicted in Fig. 3a.

Mild nonlinearities are usually not a severe problem in PLS, but this nonlinearity is quite extreme. A natural thought would be to normalize all spectra of a sample with respect to the first measured spectrum of that sample, but this did not solve the problem in this case. Rationalizing that the level of the absorbance is not indicative of the activity (as the substrate level varies from sample to sample), the measured matrix of each sample was normalized, i.e., the matrix was scaled to matrix-norm one. The effect of this normalization can be seen in Fig. 3b. The normalization helps removing the nonlinearity, and helps the algorithm focusing on the relative changes instead of the absolute changes.

For each substrate type a calibration model was made using the normalized kinetic UV/VIS data as independent and the activity as the dependent variable. The number of latent variables was determined through leave-one-out cross-validation due to the low number of samples. For CG and MIX three and four latent variables respectively was used and for EPI only two. This indicates, that the EPI system is less complex than the CG system, perhaps because the VIS area is less influenced by interferences than the UV area. Though three different models are made, the predictions can be shown together in one plot (Fig. 4). All predictions are from the cross-validations. Though the result from the cross-validation is not to be judged as the ultimate estimate of the prediction power of the model (only a test set validation can be considered so), it is the most valid result considering the low number of samples. The correlation coefficient and root mean square error of cross-validation, RMSECV, are shown in Table 2.

The weight vectors are quite informative, revealing and verifying several interesting aspects of the

Table 2
Prediction results using different calibration methods

PPO activity pred versus true	N-PLS	Unfold PLS	Tucker	PARAFAC	410 nm derivative
Corr. coef., r	0.929	0.925	0.729	0.870	0.799
RMSECV	24.2	24.8	47.9	32.6	40.4

The results are from a full crossvalidation on 3×14 samples corresponding to the three different substrate types with the first sample excluded.

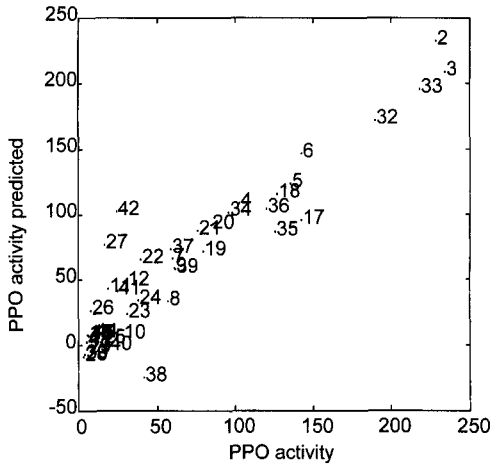

Fig. 4. PPO activity predicted from spectra versus reference PPO.

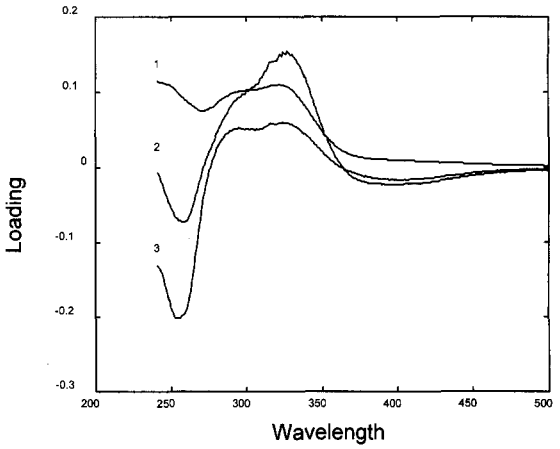

Fig. 6. Loading vectors in the spectral mode from N-PLS model the CG samples. The number corresponds to the component.

model system. Even though the color increases with time in all three systems (CG, EPI and MIX), the most (or second most) important weight vector attains a local maximum after approximately 10–20 min (shown in Fig. 5 for the CG system).

A precise relationship between the spectral changes and activity thus requires information on the spectral changes in that time span. The explanation is most likely that during the first 10–20 min the spectral changes are primarily caused by the direct effect of PPO activity. Later, the spectral changes are mainly caused by the chemical non-enzymatic polymerization of the quinons formed by the enzymatic activity. Both aspects are apparently important for relating the spectral changes to the activity of the enzyme (see Ref. [2]).

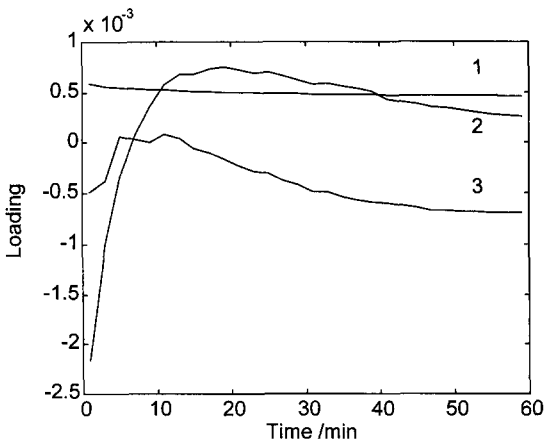

Fig. 5. Loading vectors in the kinetic mode from N-PLS model of CG samples. The number corresponds to the component.

In Fig. 6, a weight plot in the spectral mode of the CG system is shown. The weight vectors show clear resemblance with CG and some of its products (see Ref. [2]) confirming that the model is capturing the relevant spectral information.

3.3. Results from other calibration methods

Whenever several modelling strategies are investigated simultaneously, there is a danger of finding spurious results, unless great care is taken in assuring that the models are valid. This can be done statistically, by saving a test set until all decisions have been made, or by verifying the models from prior knowledge of the data, as has partly been done here. In this study several models have been investigated, and the results have been more or less equal with respect to predictions. For comparison the results will shortly be presented. Three other techniques were applied: unfold PLS, PARAFAC regression (PFR) and Tucker regression (TR). The theory of these methods will shortly be outlined.

At first however, we will mention the results of a procedure quite commonly used for describing the activity of PPO. The absorbance at one wavelength (normally between 400 and 420 nm; here 410 nm) is measured over a period. The absorbance is plotted

against the time, and the derivative at time zero is calculated, giving the initial rate of change in absorbance. This initial rate is used as a PPO assay for PPO from various fruits and vegetables (e.g., Refs. [18,19]). This approach was pursued calculating the derivative by a Savitzky–Golay algorithm [20] with a window size of seven. The best results were obtained for epicatechin, which is quite natural because epicatechin spectra change more than chlorogenic acid spectra in the visual wavelength area. However with this approach a correlation of 0.766 between the first derivative and the activity and an average calibration error calculated by full cross-validation at 30.1 (by linear regression) was obtained for the EPI system. Evidently this approach is quite inferior to the more standard chemometrical approaches, which furthermore give qualitative and quantitative information. Another way of putting it, the traditional spectrophotometrical measure of PPO activity, does not give sufficient information to predict the PPO activity (!!) as measured by the dioxygen consumption. The fact that the derivative at time zero has poor predictive power also confirms the finding that spectral changes important for predicting PPO activity happen around 10–20 min after incubation.

In unfold PLS the array of independent variables is unfolded to an $I \times JK$ matrix by concatenating the K different $I \times J$ layers horizontally. I, J and K are the dimensions of the array. This unfolded matrix can then be used in an ordinary PLS algorithm. The disadvantage of this approach is that no information is used across different orders. On the other hand, *if* the problem is not trilinear, unfold PLS might be able to describe the variations more adequately.

Unfold PLS is often mistakenly named a multiway method. Though this might to some extent be defensible, it is more likely confusing and misleading. It is a way of dealing with multiway data, but the method itself is not multiway. The main problem with unfold PLS is the little usage of multiway information in the decomposition, and the low interpretability of the solution. It is quite uncommon to see loading plots from an unfold solution, simply because these are very hard to interpret compared to multiway loading vectors. From a mathematical point of view, the unfold approach is much more complicated, than the true multilinear methods because more parameters need to be estimated, and this is also what makes the interpretation difficult. If an unfold and a multiway method are equally good (e.g., for prediction) the multiway method is preferable, because of its simplicity, which implies interpretability and robustness.

Threeway PARAFAC (parallel factor analysis) is a trilinear model, [10,21] that in some respect corresponds to principal component analysis as trilinear PLS corresponds to bilinear PLS. PARAFAC regression is simply the PARAFAC analog to principal component regression. In threeway PARAFAC each component consists of three vectors; one score vector and a loading vector in each variable direction (one spectral and one kinetic in this case). To calculate a calibration model analogous to PCR (principal component regression), the score matrix from the PARAFAC decomposition of the calibration samples is used as the independent variables in a multiple linear regression model. To predict the dependent variable for new samples, the scores of the new sample can be calculated using the loading vectors of the PARAFAC model, much as in PCR, and from the regression coefficients the dependent variable can be predicted. The main advantage of PARAFAC is that unique results are obtained under fairly mild conditions [15,22]. There is no rotational problem as in twoway analysis. If the data is indeed trilinear, the true underlying phenomena can, in principle, be found. In this case there is some evidence, however, that the data is not trilinear, because the same analytes can have different kinetic profiles depending on the sample matrix. The main disadvantage of using PARAFAC is simply that the existing algorithms are extremely slow compared to the other methods used here.

Tucker is another multiway decomposition method that is beginning to show promising results in chemometrics [10,23–25]. Apart from Ref. [25] no application of Tucker in calibration problems has to our knowledge occurred, though the principle is as simple as in all other decomposition methods.

The Tucker model is conceptually more sophisticated than PARAFAC and N-PLS, in that the number of loading vectors in each order need not be the same. Furthermore a loading vector is not only related to *one* vector in each of the other modes, but can be related to all other loading/score vectors of the other modes.

To exemplify this, a hypothetical twoway example will show the basic idea of a Tucker model. Consider a matrix of chromatographically obtained spectra of a sample containing two analytes with different spectra but the same chromatographic profile. Looking at any column (wavelength) one will see the same picture, namely the chromatogram, and looking at a row (retention time) one will see either a sum of the spectra of the two analytes or noise if outside the peak. Without prior knowledge it is not possible to decompose this matrix into the two underlying spectra, but a good representation of the data would be the outer product of one column vector, i.e., the chromatographic profile and a weighted sum of two row vectors, i.e., the underlying spectra. This can be written as

$$\mathbf{pCS}',$$

p being the chromatographic profile, **S** the two by the number of wavelengths matrix of the spectra and **C** a one by two matrix containing the weighting of the spectra. The elements **C** can be regarded as a kind of singular values, and is called the *core matrix*.

In threeway analyses one would obtain three sets of loadings instead of two, and the core matrix would be a cube with dimensions given by the number of factors extracted in each direction. If the number of loading vectors in all three directions is the same, and all elements of the core cube are zero except for the superdiagonal, the solution equals the PARAFAC solution; hence PARAFAC is a restricted version of the Tucker model. If the elements in the non-superdiagonal are not zero, these can be interpreted in the following way: *If* the element i, i, k is large and much larger than i, i, i, this means that the ith loading in the first order, the ith loading in the second order, and the kth loading in the third order constitutes a rank-one model, that is more descriptive of the data than the rank-one model given by the ith loading of the first order the ith loading of the second order and the ith loading of the third order.

The essential fact of the Tucker model is that it is more flexible than PARAFAC and N-PLS and it is more restricted than unfold methods. So the Tucker model is in-between these two types of models. Which model is best depends on the data structure, and is not always obvious, but must be tested on the specific problem.

Like PARAFAC regression Tucker regression can be performed by regression using the scores (loadings of the first mode) as independent variables. For a new sample the scores can be calculated by the loadings of the second and third order and the core array. This is done as calculating the scores in the iterative Tucker algorithm, only now the algorithm is not iterative because only the scores are unknown.

The results of unfold PLS, PARAFAC and Tucker are shown with the N-PLS results and the results from the traditional method using the first derivative of the kinetics at 410 nm in Table 2.

The results using these different methods clearly show that the multiway PLS algorithm is advantageous. Though unfold PLS gives almost equally good predictions the model is a lot more complex. Each loading vector in an unfold model consists of several thousands parameters, while in the true multiway methods only a few hundred parameters are estimated. As shown in Results and Discussion the interpretation of the threeway model is quite easy. As a comparison one could try to interpret the main loading vector from unfold PLS, which is shown in Fig. 7. Though a general structure is observed, there are also deviations, that are somewhat difficult to interpret and quantitate in comparison with the threeway model. Notice for example the first part of the unfold loading vector. There are phenomena here that are quite different from the remaining part. Though interesting in itself, it is apparently not essential for

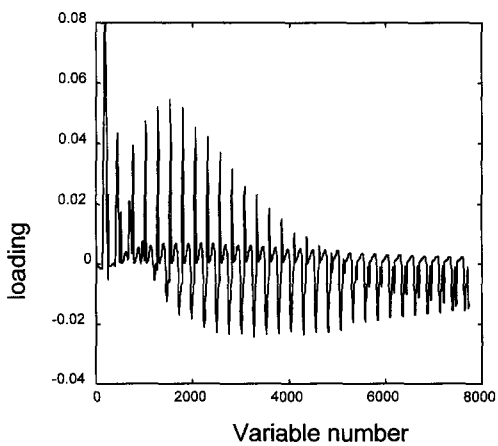

Fig. 7. The first loading vector from an unfold PLS model of the CG samples.

predicting the activity as the N-PLS does not contain any such discrepancies yet still predicts well. Compared to the N-PLS solution it is harder to interpret the loading vector, in essence simply because the unfold model contains more parameters.

PARAFAC gives distinctly poorer results than N-PLS and unfold PLS. The reason might be that there is some degree of non-trilinearity in the data, because equal species might have different kinetic profiles under different circumstances. This is confirmed by rank analysis on unfolded matrices from the calibration cube. If the data is trilinear one would most often expect equal or almost equal bilinear rank of the unfolded calibration cube, no matter which way it is unfolded. In this case however, the rank was equal when the sample or the spectral mode was kept intact, but if the kinetic mode was made the intact mode, the rank was almost twice as high. The reason that N-PLS is not as influenced by this disagreement between the model and the data, is most likely that the latent variables are not calculated simultaneously as in PARAFAC. Therefore the most relevant, and to some extent most trilinear, variations are allowed to influence the model more than the nonsystematic or nontrilinear variations. The PARAFAC model includes all variations, also nontrilinear, in order to make the least squares model. The Tucker model did not give good results. Somehow the flexibility offered by the Tucker model is not appropriate for this data set.

4. Predicting chromatographic peaks from kinetic UV/VIS spectra

4.1. Mathematical model

The dependent variable in this problem is a five-dimensional vector containing the relative peak area of either chlorogenic acid or epicatechin measured by HPLC after five different incubation times (Table 1). As in the previous example the independent variables are the kinetic UV/VIS spectra corresponding to the conditions for the HPLC analysis. The model used in this section is the same as described for predicting activity from UV/VIS spectra, except that in this case **Y** is multivariate, so the trilinear PLS2 algorithm is used.

4.2. Results and discussion

As before all sample matrices (spectra × kinetic) of the independent variables were normalized to reduce nonlinearities, and as before it was not possible to incorporate the three different substrate types into one model. In this case two models were made; one for predicting CG in the CG and MIX samples and one for predicting EPI in the EPI and MIX samples. Full cross-validation was used.

Due to the low number of samples — ten in each model — it was decided to include all samples in the calibration even though some were potential outliers. Altogether the circumstances were such so that only indications could be hoped for by this model. It is unrealistic to hope that all relevant variations (interferences from substrate, O_2 and CO_2) can be captured by a model built from only ten samples. Taking into account these difficulties, the model obtained works reasonably. For CG RMSECV was 0.510 and the correlation coefficient 0.707 (two components). For EPI the result was an RMSECV of 0.169 and a correlation coefficient of 0.686 (one component). The errors are large, but from the predictions (Fig. 8) one sees, that the model actually captures the main variations both with respect to the general level of the substrate consumption and also with respect to the time dependent variations in the substrate consumption (see Ref. [2]).

Though the model cannot predict the substrate consumption precisely from the data used here, the result implies the important fact, that substrate consumption can indeed be predicted from the spectral variations in a model system. Therefore it is possible to replace the tedious and time-consuming HPLC analysis with a direct spectral analysis of the sought PPO system. Clearly a larger calibration set is necessary, and if only one substrate type is to be modelled the predictions would most probably be better.

4.3. Results from other calibration methods

Only the unfold PLS was tried as an alternative in this case, as the data almost beforehand was known to be to complex to model properly considering the low number of samples. The result of the unfold PLS was an RMSECV for CG at 0.506 (two components) and for EPI at 0.169 (one component). The results are quite similar to the ones obtained with N-PLS, and the

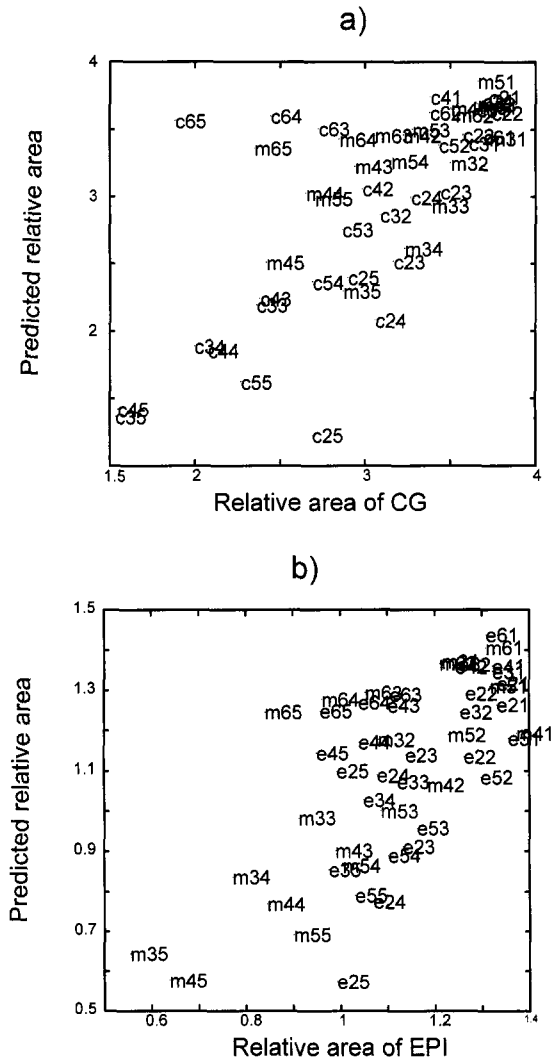

Fig. 8. Cross-validated predictions of CG (a) and EPI (b) from kinetic spectra. The letter indicates the substrate type (e-EPI,c-CG, m-MIX). Sample c43 is the third peak of the fourth CG sample etc.

increased complexity of the bilinear PLS therefore does not offer any advantages for this data set.

5. Predicting activity from experimental design. Analysis of variance

5.1. Mathematical model

The data in this example constitute a full five-factor factorial design, but due to very unsatisfactory results by standard ANOVA techniques (see later) other approaches had to be pursued. The data are treated in two different ways (see Table 2). In the PARAFAC model, the data are interpreted as a multiway array of activities, specifically a fiveway array. The five different modes/ways are: O_2 (dimension five), CO_2 (dimension three), pH (dimension three), temperature (dimension three), and substrate type (dimension three). The *ijklm*th element of the fiveway array contains the activity at the ith O_2 level, the jth CO_2 level, the kth level of pH, the lth level of temperature, for the mth substrate type. The fiveway array is tried depicted in Fig. 9.

For the standard multivariate models (locally weighted regression, PLS and neural networks) the data are arranged in two data tables: A 405×1 vector of dependent variables (PPO activity) and a 405×6 matrix of independent variables each column corresponding to a factor, such that the first factor describes the level of O_2, the second CO_2, the third pH, the fourth temperature, the fifth the level of CG and the sixth the level of EPI. In this case the data is thus considered to be twoway, each factor constituting a variable. The data structure is shown in Fig. 10.

The PARAFAC model has shortly been described above. For a more thorough description the reader is referred to Refs. [14,15,26]. In this application the model is used for analysis of variance, and the applied model is a fiveway model. This means that one

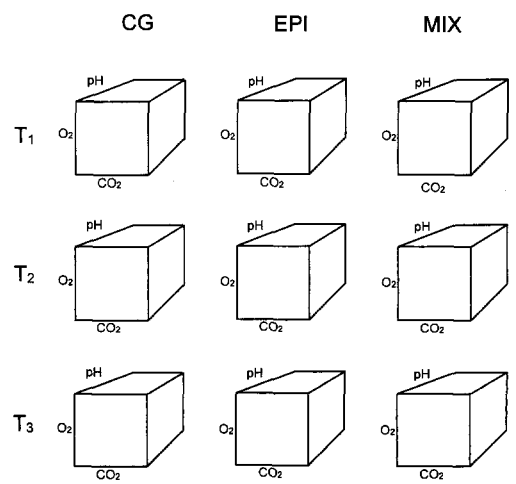

Fig. 9. A graphical representation of the fiveway array of activities.

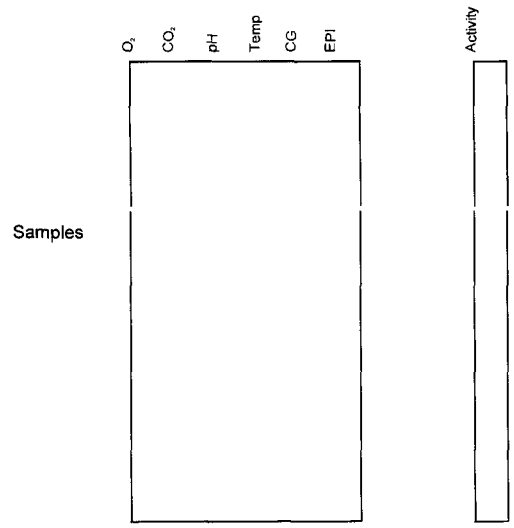

Fig. 10. A graphical representation of the two calibration arrays used in the bilinear models.

component consists of five loading vectors: **a**, **b**, **c**, **d**, and **e**, corresponding to the modes of O_2, CO_2, pH, T, and substrate.

The use of PARAFAC for analysis of variance is rare [27]. However, the use of PCA and related methods for analyzing twoway interactions has been known for several years (see Refs. [28,29] and references therein). The advantage of using PARAFAC for ANOVA is in the way interaction terms are modelled. In a standard ANOVA an interaction between three factors (A, B and C) would be estimated as abc_{ijk}, while in a trilinear model, this effect would be estimated as $a_i b_j c_k$ or as a sum of such expressions if more PARAFAC components are estimated, i.e., the interaction is not only estimated as a whole, but is modelled as a multiplicative effect of the three different factors. *If* the multiplicative model is appropriate, the applied restriction ($a_i b_j c_k$ instead of merely E_{ijk}) will give a more interpretable model. This is comparable to the well-known result of performing PCA/PARAFAC decomposition of, e.g. spectral data.

5.2. Results and discussion

5.2.1. Preprocessing

When analyzing the fiveway array of activities by PARAFAC several issues must be decided. Preprocessing is quite important for obtaining the right model, but the theory of preprocessing is quite incomplete with respect to ANOVA-like data [30]. Consider a one-component fiveway PARAFAC model. This is given as

$$x_{ijklm} = a_i b_j c_k d_l e_m,$$

disregarding noise contributions. If however the data is meancentered within a certain mode, the second for example, the model is now

$$x_{ijklm} = \bar{b}_{iklm} + a_i b_j c_k d_l e_m,$$

$\bar{b}_{iklm}$ being the mean of the $iklm$th column.

Scaling does not change the underlying model, but merely the way the parameters are computed, that is, how much weight is put on different variables. As can be imagined many combinations of scaling and meancentering, can greatly affect the outcome of the model. However, deciding on the proper preprocessing is difficult in this case. No theoretical results can guide on what kind of model is most appropriate. As a consequence, it was chosen to try the simplest possible model, that is, a model on the raw data. It turned out that this model was better than any single-centered model. The reason might very well be, that any mean value is very poorly estimated in this data set because most of the modes have only three elements in each column (centering is done on individual columns/vectors of the array in PARAFAC). Some heteroscedasticity was present in the dioxygen mode, and a model was made scaling this mode. This model, however, had poorer predictive power, than the non-scaled model, simply because down-weighting the uncertain elements also means that the prediction errors of these increase. Hence, a PARAFAC model on the raw data turned out to give the best results.

5.2.2. Validation

As this application of PARAFAC is different from traditional analysis of variance we will show several different types of validation procedures.

To choose the number of components, a PARAFAC model was made using the first set of the two replicate sets instead of using the mean of these. The model from this analysis given by the loadings $\boldsymbol{A}$, $\boldsymbol{B}$, $\boldsymbol{C}$, $\boldsymbol{D}$ and $\boldsymbol{E}$ was compared to the other replicate set. The number of components, F, was chosen

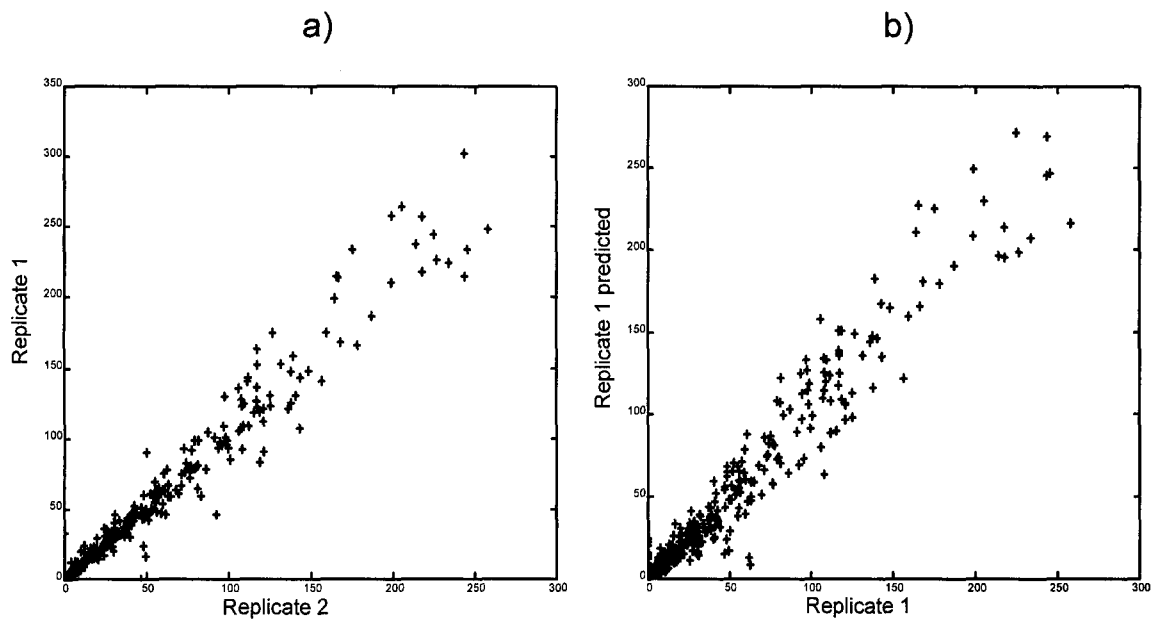

Fig. 11. (a) Replicate 1 versus replicate 2. (b) Replicate 1 predicted from model of replicate 2 versus repicate 1.

to minimize the predicted residual sum of squares, PRESS, calculated as

$$\text{PRESS} = \left[\left(\sum_{f=1}^{F} a_{if} b_{jf} c_{kf} d_{lf} e_{mf}\right) - x_{ijklm}\right]^2, \quad (5)$$

x_{ijklm} being the $ijklm$th element/activity of the replicate set not used to build the model. One component gave the lowest prediction error, which furthermore was in the neighborhood of the intrinsic error of the reference value. In Fig. 11a the reference values from the first replicate set are plotted against the second and in Fig. 11b the first set of replicates is plotted against the activities obtained from the model made from the second set of replicates. The root mean square error between the two sets of replicates is 11.88 and 13.39 between the predictions and the replicates. Most of the prediction error (85%) can

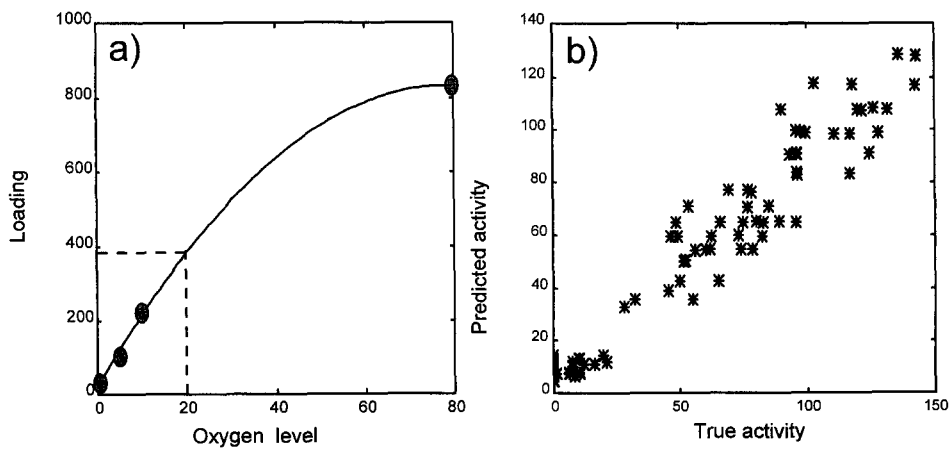

Fig. 12. (a) The dioxygen loadings (●) calculated without samples with 20% dioxygen and the estimated relation between dioxygen and loading (the line). (b) The prediction of activity of 20% dioxygen samples from the estimated loading.

hence be assigned to the error in the true values. Apparently, the 405 samples can be modelled almost completely by a model consisting of 17 (5 + 3 + 3 + 3 + 3) parameters!

A one-component solution was also found from a test set procedure of a fractional factorial design where half the elements of the fiveway array were eliminated. 202 of the 405 elements were eliminated by simply stringing out the array to a 405 × 1 vector, eliminating every second element and refolding the vector to the fiveway array. Thereby every non-missing element is surrounded by missing elements, and every missing element is surrounded by non-missing elements in the fiveway array. For the remaining approximately half of the data, a PARAFAC model was built with an algorithm, that handles missing data. With the model, the predictions of the left-out samples were obtained for both a one- and a two-component PARAFAC model. RMSEP for one component was 12.55, while for two components an RMSEP of 51.92 was found. This validation procedure also shows that PARAFAC can easily handle missing data; in this case half of the elements were systematically missing.

From the PARAFAC loadings it is possible to predict the effect of any level of the factors investigated. Plotting the loading vector of e.g., dioxygen against the dioxygen level one gets a graph, from which the effect of any dioxygen level can be read. To validate this, a PARAFAC model was made leaving out all samples with 20% dioxygen. The loadings of the dioxygen mode determined without the 20% level is shown versus the level in Fig. 12a. Also shown is a quadratically estimated relation between the dioxygen level and the loading. From this curve the loading at the 20% level can be estimated as 383. From this value and the loadings of the remaining modes the 27 (1 × 3 × 3 × 3) left-out samples are predicted with a root mean square error of 13.11 (shown in Fig. 12b). This shows, that from the PARAFAC model a completely general model is obtained showing the effect of each factor as simply a loading vector.

5.2.3. *The final model*

The outcome of the PARAFAC model is extremely simple. Enzymatic activity in various experi-

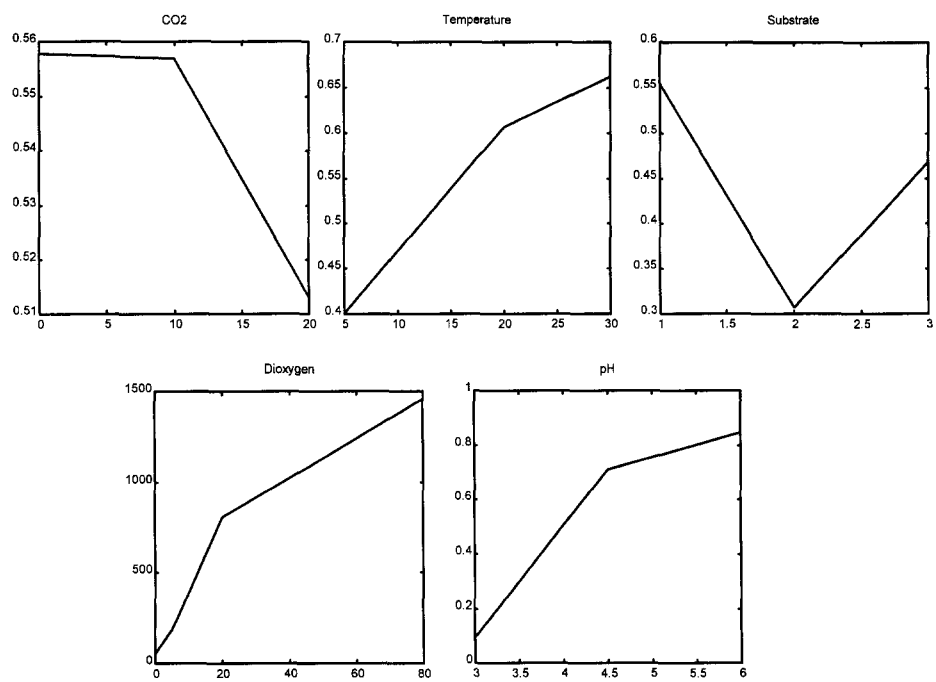

Fig. 13. The loadings of the PARAFAC model plotted against the factor levels.

mental designs can be described by a five-parameter multiplicative model

$$x_{ijklm} = a_i b_j c_k d_l e_m. \quad (3)$$

It is very interesting, that the only thing necessary to model the data is a fiveway interaction term. No additive terms at all are necessary. For any of the modes a plot of the loading vector will immediately show how changing the factor of that mode will affect the enzymatic activity. The factors are shown in Fig. 13. From these plots it is easy to see which conditions are most appropriate for keeping the PPO activity, and therefore the undesirable color formation, low. Controlling, e.g., the dioxygen level will be a very efficient way of keeping the PPO activity low, while only small effects can be obtained by controling the CO_2 level. Here certain details will be a little further investigated, some of which are discussed more Ref. [2].

The loadings of the substrate mode show that chlorogenic acid is a much better substrate for PPO than epicatechin, and that the mixture of both substrates is not merely the sum of the activities of the two. This supports the hypothesis, that CG acts primarily as substrate for PPO, while EPI to a larger degree is involved with the non-enzymatic polymerization [31]. This important finding is further discussed in Ref. [2].

For the CO_2 mode the loading vector show that 20% CO_2 decreases the PPO activity slightly (notice the scale on the figure), while there is only little difference between the loadings of the 0 and 10% level. It is technologically interesting to nail down if this small difference is significant or merely coincidental. To test this, the two replicate sets were each split in two sets, giving a total of four sets all with nearly half of the elements missing. As all elements of the replicates are measured independently, four independent data sets were now available. A model was made from each of the sets, and the CO_2 loading vector was normalized. The residuals of the two levels were found to be homoscedastic and a t-test was made to test if the 20% CO_2 loading could be equal to or higher than the loading of the 10% level. On the basis of these four estimates of the loadings, the hypothesis was rejected (0.00005% significance level). By ANOVA it was not possible to detect any difference in the effect of CO_2 on the 20 and 10% level [2], but from this result, it is clear that changing the CO_2 level does have a small effect.

6. Results from other calibration methods

Analysis of variance (ANOVA) was carried out in SAS using the full replicated design allowing all interactions, and the five factors also used in the PARAFAC model. The result was difficult to interpret as 26 effects/interactions were found to be significant including many three- and fourway interactions. The proposed ANOVA model is given below,

$$\begin{aligned}\text{activity}_{ijklm} &= a_i + b_j + c_k + d_l + e_m + ab_{ij} + ac_{ik} + ad_{il} \\ &\quad + ae_{im} + bc_{jk} + bd_{jl} + be_{jm} + cd_{kl} \\ &\quad + ce_{km} + de_{lm} + abc_{ijk} + abd_{ijl} + abe_{ijm} \\ &\quad + acd_{ikl} + ace_{ikm} + abcd_{ijkl} + abce_{ijkm} \\ &\quad + ade_{ilm} + abde_{ijlm} + cde_{klm} + acde_{iklm},\end{aligned}$$

Interestingly the fiveway interaction was not significant. The fiveway interaction was the *only* effect used in the PARAFAC model, so the two methods point to quite different solutions. Using half the data for calibration a regression model was made with all significant effects using the combined multiple linear regression and ANOVA method in SAS. The result was a fair prediction (Table 3) using several hundreds of parameters. Comparing the error, 35.3, with an error of 12.6 from PARAFAC which furthermore only uses 17 parameters, there is no doubt that the PARAFAC model is advantageous in this case. Bet-

Table 3
RMSEP of an independent test set of 203 samples

PCR (5)	33.6
PLS (3)	33.5
QuadPLS (5)	24.4
CubicPLS (4)	24.3
NN (3)	24.7
LWR (5)	16.6
PARAFAC (1)	12.6
ANOVA	35.3

The numbers in parentheses are the number of latent variables/hidden nodes found by cross-validation on the calibration set.

ter ANOVA models could possibly be obtained, but the important aspect in this context is that ANOVA and PARAFAC points at extremely different models of the data. ANOVA suggests 26 effects while PARAFAC suggests one. PARAFAC furthermore offers straightforward generalization of the effects to other levels of the factors, than the ones explicitly used in the experiment.

The six factors varied in the experiment (O_2, CO_2, pH, temperature, CG, and EPI) can be considered as a multivariate data set with six independent variables, and the activity being the dependent variable. Different multivariate calibration techniques were tried to fit the data: Locally weighted regression [32], ordinary linear PLS, PLS with quadratic and cubic [33] [34], inner relations, and ordinary feedforward neural networks[35,36] with one and two hidden sigmoidal layer and one to five hidden neurons in each layer. Most of these methods have been well described in the literature, so only the quantitative results will be shown together with a few comments (Table 3). The test and calibration set was the same in all cases (every second sample belonging to the test set etc.), and parameters such as the number of latent variables was determined from the calibration set alone, by a 10 segmented systematic cross-validation procedure. The conditions for the different methods was as follows:

LWR. In each prediction the 25 closest samples were used for the local regression (weighted linear regression). The weighting of a sample was based on the weight $(1 - u^3)^3$, where u is the distance of the sample relative to the sample furthest away from the new sample to be predicted. The distance was calculated using the Mahalanobis distance of a 5 component PCA model.

PCR. The independent variables were autoscaled.

PLS. The independent variables were autoscaled.

Quadratic and cubic PLS. The independent variables were autoscaled. Latent variables were calculated dimensionwise and only polynomials of the score itself was used.

Ordinary feedforward neural networks. The neural networks were trained by backpropagation and stopped when a predefined subset gave a minimal prediction error. The best result of ten runs was used. A neural network with one hidden layer was chosen, as two hidden layers did not improve the error.

The important result of the alternative calibration methods is that they do not work as well as the PARAFAC model, neither with respect to predictions nor interpretability. The nonlinear relationship between PPO activity and the factors varied, make it difficult for the linear multivariate calibration methods to work. PLS and PCR are equally bad. A little better is PLS with quadratic and cubic inner relation, and surprisingly neural networks are not better than nonlinear PLS. Neural networks are more flexible especially when several hidden layers are used, but apparently the flexibility offered is not sufficient for the problem at hand. LWR gives much more precise predictions than any of the beforementioned methods.

Knowing the most probable relation between the factors and PPO activity from the PARAFAC model, it is interesting, that none of the methods can properly describe the variations. Only LWR, which requires many samples comes close to the PARAFAC model.

7. Conclusion

We have shown that multiway methods have potential in very different types of problems. Applying the right model to the data can sometimes give results which might otherwise not have been thought to be obtainable, c.f., the traditional model relating absorbance kinetics to PPO activity. Two examples of the application of N-PLS and one of PARAFAC have been shown and compared to other techniques. Not only are the methods competitive with respect to the primary goal, but all of them give quantitative and qualitative information on the models/data that would not otherwise have been obtained. For the N-PLS model relating kinetic UV/VIS spectra to PPO activity a good model was obtained with a reasonably low prediction error, but furthermore with loading and score vectors giving important information on how the spectra relate to the activity. The calibration of HPLC results was not impressing but considering the many variation induced in the few samples this is not surprising. The important thing is, that we now have reason to believe that a better calibration model could be build, from an appropriate data set. The PARAFAC model of the PPO activity is a good example of an alternative to traditional ANOVA. It

gives a very interpretable and robust model of the data; a model that is quite different from the ANOVA model.

Acknowledgements

R.B. is grateful for support and inspiration from and funds to Professor Lars Munck from Nordic Industry Foundation project P93149 and the FØTEK foundation. Lars Nørgaard is thanked for numerous suggestions during this work.

References

[1] M.V. Martinez and J.R. Whitaker, Trends Food Sci. Technol. 6 (1995) 195–200.
[2] H. Heimdal, L.M. Larsen, L. Poll and R. Bro, J. Agric. Food Chem., submitted.
[3] F.C. Richard-Forget, M. Rouet-Mayer, P.M. Goupy, J. Philippon and J.J. Nicolas, J. Acgric. Food Chem. 40 (1992) 2114–2122.
[4] H. Heimdal, L.M. Larsen and L. Poll, J. Agric. Food Chem. 42 (1994) 1428–1433.
[5] B.M. Wise and N.B. Gallagher, PLS_Toolbox, Vol. 1.5, Eigenvector Technologies, 4154 Laurel Drive, Richland, WA 99352 (1995).
[6] H. Martens and T. Næs, Multivariate calibration (John Wiley and Sons, Inc., Chichester, 1989).
[7] R. Bro, J. Chemom. 10 (1996) 47–61.
[8] A.K Smilde, Chemom. Intell. Lab. Syst. 5 (1992) 143–157.
[9] H.A.L. Kiers, Psychometrika 56 (1991) 449–470.
[10] P. Geladi, Chemom. Intell. Lab. Syst. 7 (1989) 11–30.
[11] E.R. Malinowski, Factor analysis in chemistry (John Wiley and Sons, Inc., New York, 1991).
[12] M.B. Seasholz and B.R. Kowalski, J. Chemom. 6 (1992) 103–111.
[13] J.B. Kruskal, Proc. Symp. Appl. Math. 28 (1983) 75–104.
[14] R.A. Harshman and M.E. Lundy, Data Preprocessing and the Extended PARAFAC Model, in: Research Methods for (1993) 508–517. Mulitmode Data Analysis, ed. H.G. Law, C.W. Snyder, J.A. Hattie and R.P. McDonald (Praeger, New York, 1984).
[15] R. Bro, PARAFAC: Tutorial and Applications, Chemom. Intell. Lab. Syst., submitted.
[16] J. Mandel, Am. Stat. 36(1) (1982) 15–24.
[17] H. Martens and T. Næs, Multivariate Calibration by Data Compression, in: Near Infrared Technology in the Agricultural and Food Idustries, ed. P. Williams (St. Paul, MN, 1987).
[18] D. Ke, M.E. Saltveit Jr., Plant. Physiol. 88 (1988) 1136–1140.
[19] S. Fujita, T. Tono and H. Kawahara, J. Sci. Food Agri. 55 (1991) 643–651.
[20] A. Savitzky and M.J.E. Golay, Anal. Chem. 36 (1964) 1627–1639.
[21] R. Harshman, P. Ladefoged and L. Goldstein, J. Acoust. Soc. Am. 62 (1977) 693–707.
[22] S. Leurgans, R.T. Ross and R.B. Abel, SIAM J. Matrix Anal. Appl. 14 (1993) 1064–1083.
[23] A.K. Smilde, Y. Wang and B.R. Kowalski, J. Chemom. 8 (1994) 21–36.
[24] H.A.L. Kiers and A.K. Smilde, J. Chemom. 9 (1995) 179–195.
[25] L. Nørgaard, Spectral Resolution and Prediction of Slit Widths in Fluorescence Spectroscopy by Two and Three Way Methods, J. Chemom., submitted.
[26] R.A. Harshman and M.E. Lundy, The PARAFAC Model for Three-Way Factor Analysis and Multidimensional Scaling, in: Research Methods for Mulitmode Data Analysis, ed. H.G. Law, C.W. Snyder, J.A. Hattie and R.P. McDonald (Praeger, New York, 1984).
[27] J.R. Kettenring, Proc. Symp. Appl. Math. 28 (1983) 105–139.
[28] A. Aastveit and H. Martens, Biometrics 42 (1984) 829–844.
[29] H. Martens, L. Izquierdo, M. Thomassen and M. Martens, Anal. Chim. Acta 191 (1986) 133–148.
[30] PM Kroonenberg, Three-Mode Principal Component Analysis (DSWO Press, Leiden, 1983) reprint 1989.
[31] V. Cheynier, N. Basire and J. Rigaud, Agric. Food Chem. 37 (1989) 1069–1071.
[32] T. Næs, T. Isaksson and B.R. Kowalski, Anal. Chem. 62(7) (1990) 664–673.
[33] S.D. Oman, T. Næs and A. Zube, J. Chemom. 7 (1993) 195–212.
[34] S. Sekulic, M.B. Seasholtz, Z. Wang, B.R. Kowalski, S.E. Lee and B.R. Holt, Anal. Chem. 65(19) (1993) 835A–845A.
[35] G. Kateman, Chemom. Intell. Lab. Syst. 19 (1993) 135–142.
[36] B. Walczak and W. Wegscheider, Anal. Chim. Acta 283