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Simultaneous spectrophotometric determination of maltol, ethyl maltol, vanillin and ethyl vanillin in foods by multivariate calibration and artificial neural networks

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Abstract

Maltol (MAL), ethyl maltol (EMA), vanillin (VAN) and ethyl vanillin (EVA) are food additives, and they have well defined UV spectra. However, these overlapped seriously, and it is difficult to determine them individually from their mixtures without a pre-separation. In this paper, chemometric approaches were applied to resolve the overlapping spectra and to determine these compounds simultaneously. The analysis of these four compounds was facilitated by the use of an orthogonal array data set consisting of absorption spectra in the 200–350 nm ranges obtained from a calibration set of mixtures containing these compounds. With this dataset, seven different chemometric models were built, such as classical least squares (CLS), principal components regression (PCR), partial least squares (PLS), and artificial neural networks (ANN). These chemometric models were then tested by the use of a validation dataset constructed from synthetic solutions of these four compounds. The analytical performance of these chemometric methods was characterized by relative prediction errors (RPE) and recoveries. The proposed methods were successfully applied to the analysis of commercial food samples. It was found that the radial basis function artificial neural networks (RBF-ANN) gave better results than other chemometric methods. PLS, PCR, DPLS, and DPCR also give satisfactory results, while CLS and DCLS perform poorer. It was also found that there was no advantage to pre-treat spectra by taking derivatives. The four compounds, when taken individually, behaved linearly in the 1.0–20.0 mgl^{-1} concentration range, and the limits of detection (LOD) for MAL, EMA, VAN and EVA were 0.39, 0.56, 0.49 and 0.38 mgl^{-1}, respectively.

Keywords: Spectrophotometry; Multivariate calibration; Artificial neural networks; Maltol; Ethyl maltol; Vanillin; Ethyl vanillin

1. Introduction

Maltol (MAL), ethyl maltol (EMA), vanillin (VAN) and ethyl vanillin (EVA) are important food additives as flavor enhancers. Their molecular structures are as shown in Scheme 1. These compounds are widely used to contribute to the fragrance of commercial foods such as candies, cookies, chocolate and beverages (Heath, 1978; Hui, 1992). Although these compounds can enhance the scent of foods, they are synthetic perfumes and food additives. If large amounts of these flavor enhancers are ingested, they cause headaches, nausea and vomiting, and could affect liver and kidney functions (Han, 2002). Consequently, it is important to determine their contents in foods.

The analytical methods for determination of MAL, EMA, VAN and EVA, include gas chromatography–mass spectrometry methods (GC–MS) (Adahchour,

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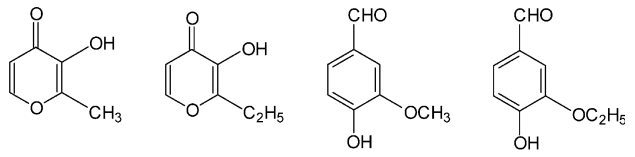

Scheme 1.

Vreuls, van der Heijden, & Brinkman, 1999; Clark & Bunch, 1997), high performance liquid chromatography methods (HPLC) (Portela, Baluguera, Goicolea, & Barrio, 1996), UV–Vis spectrometry methods (UV) (Li, Jiang, Mao, & Shen, 1998; Cheng, Zhang, & Lin, 2000), and electrochemical methods (EC) (Agui, Lopez-Guzman, Gonzalez-Cortes, Yanez-Sedeno, & Pingarron, 1999; Luque, Luque-Perez, Ryos, & Valcarcel, 2000), have been reported.

The chemometric methods are an effective way to analyze simultaneously several analytes (Galeano Diaz, Guiberteau, Ortiz Burguillos, & Salinas, 1997). The abilities of different chemometric methods to resolve mixtures of different compounds with overlapped spectra have been widely utilized. The main advantage of multicomponent analysis by chemometric methods (multivariate calibration and artificial neural networks in this work) is the rapid determination of the components in mixtures avoiding a prior separation, which is generally necessary owing to the overlapped signals. Recently, the chemometric methods such as classical least squares (CLS), principal components regression (PCR), partial least squares (PLS), artificial neural networks (ANN) have found increasing applications for multicomponent determination (Ni & Gong, 1997; Duran-Meras, Munoz-de-la Pena, Espinosa-Mansilla, & Salinas, 1993; Adsu, Bozdogan, & Kunt, 1998; Ventura, Silva, & Perez-Bendito, 1995). These methods are effective in spectrophotometric analysis, because they can improve the performance and application of the analytical method with the use of simultaneous analysis of several spectra. Generally, when applying chemometrics methods for prediction, a set of calibration samples with known compositions is first prepared and the absorption spectral measurements are carried out; then the mathematical models are established by processing the measured spectral data. Subsequently, the models are used for the prediction of unknown samples under the same experimental conditions.

In this work, the simultaneous determination of the mixtures of MAL, EMA, VAN and EVA by UV–Vis spectrophotometry was carried out with the use of chemometrics methods (CLS, PCR, PLS and ANN). Recently, it has been shown that the transformation of the raw spectra to their derivatives is a successful data pre-treatment method, and its usefulness for spectrophotometric analysis has been evaluated in several studies (Kompany-Zareh, Massoumi, & Pezeshk-Zadeh, 1999; Ni & Jin, 1999). This pre-treatment of spectral data was also investigated in this work. All the mentioned chemometrics methods were applied for the analysis of synthetic samples and several commercial food samples, and the results obtained are compared.

2. Materials and methods

2.1. Chemicals

All solutions were prepared with analytical-grade reagents (Shanghai Reagents Company), and doubly distilled water was used throughout. Stock solutions of MAL, EMA, VAN and EVA (1.00 $g l^{-1}$) were prepared by dissolving each of the crystalline compounds in 95% ethanol. Working solutions of 250 $mg l^{-1}$ were prepared by dilution with distilled water before use. Britton–Robinson buffer solution (pH 2.87) was prepared from phosphoric acid, acetic acid, boric acid and sodium hydroxide (Zhu, Wang, & Lu, 1999).

2.2. Apparatus

Spectrophotometric measurements were made on a UV-2501PC spectrophotometer (Shimadzu), equipped with a thermostat (TB-85, Shimadzu). The pH of the solutions was measured with a pH meter (SA-720, Orion).

The data were processed on a Pentium IV computer with programs written in MATLAB 6.0 (Mathworks).

2.3. Analytical procedure

Suitable amount of working solutions of MAL, EMA, VAN and EVA, or their mixtures, were transferred to 25 ml volumetric flasks followed by the addition of 5.0 ml of the Britton–Robinson buffer solution (pH 2.87), diluted to the mark with the doubly distilled water, and mixed well. The absorbance spectrum of each solution was measured with respect to a reagent blank between 200 and 400 nm at a 1 nm interval, and all these data were recorded and used for calculations.

2.4. Linear calibration models for maltol, ethyl maltol, vanillin and ethyl vanillin

A set of eight samples with different analyte concentrations was prepared for each flavor enhancer, and the absorbance measurements were carried out as described in Section 2.3. Each peak of these compounds has a good linear relationship with the concentrations. Parameters of the models of each compound are summarised in Table 1. It can be seen that good correlation

Table 1
Parameters of linear calibration models for maltol, ethyl maltol, vanillin and ethyl vanillin separately

Parameters	Maltol (273 nm)	Ethyl maltol (274 nm)	Vanillin (278 nm)	Ethyl vanillin (279 nm)
Sample number (n)	8	8	8	8
Linear range (mgl^{-1})	1.0–20.0	1.0–20.0	1.0–20.0	1.0–20.0
Slope (lmg^{-1})	0.0720	0.0664	0.0682	0.0642
Intercept ($\times10^{-3}$)	7.8	−2.4	5.5	8.8
s_b ($\times10^{-4}$)[a]	4.5	6.2	5.9	4.4
s_a ($\times10^{-3}$)[a]	5.4	7.3	6.9	5.2
Correlation coefficient	0.999	0.999	0.999	0.999
Detection limit (mgl^{-1})[b]	0.39	0.56	0.48	0.38

[a] s_a is the SD of the intercept, and s_b is the SD of the slope.
[b] The detection limits for all compounds were calculated according to Millers' method (Miller and Miller, 2000).

coefficients are observed at main peak wavelengths for each compounds.

2.5. Prediction of four flavor enhancers in synthetic mixtures

The orthogonal array design method was used to optimize the calibration set in this work. Application of a four-level orthogonal array design, denoted by $OA_{16}(4^5)$ (Lan, Wong, Ni, & Sin, 1994), showed that 16 samples were required with the compositions as shown in Table 2. Another 16 synthetic mixtures containing these four compounds, mixed in randomly selected ratios (Table 3), were prepared for validation. The concentrations of the enhancers in the latter set were estimated by processing the absorbance data with the aid of the calibration models, including CLS, PCR, PLS, DCLS, DPCR, DPLS (D = derivative transformation as spectral pre-treatment), and RBF-ANN. To investigate the uncertainty of prediction for each calibration method, the relative prediction error (RPE) was calculated (Otto & Wegscheider, 1985). The RPE for a single component in mixtures can be formulated as

$$\mathrm{RPE_S} = \left[\frac{\sum_{i=1}^{n}\left(c_{\mathrm{pred},i,j} - c_{\mathrm{real},i,j}\right)^2}{\sum_{i=1}^{n}\left(c_{\mathrm{real},i,j}\right)^2}\right]^{0.5}, \quad (1)$$

and the RPE for all components can be formulated as

$$\mathrm{RPE_T} = \left[\frac{\sum_{i=1}^{n}\sum_{j=1}^{m}\left(c_{\mathrm{pred},i,j} - c_{\mathrm{real},i,j}\right)^2}{\sum_{i=1}^{n}\sum_{j=1}^{m}\left(c_{\mathrm{real},i,j}\right)^2}\right]^{0.5}, \quad (2)$$

where $c_{\mathrm{real},i,j}$ indicates the concentration of jth component in ith mixture and $c_{\mathrm{pred},i,j}$ is its estimate found by chemometric methods.

Table 2
Composition of the calibration samples

Samples	Concentration (mgl^{-1})			
	Maltol	Ethyl maltol	Vanillin	Ethyl vanillin
1	1.00	1.00	1.00	1.00
2	1.00	3.00	2.00	2.00
3	1.00	5.00	4.00	4.00
4	1.00	7.00	7.00	7.00
5	3.00	1.00	2.00	4.00
6	3.00	3.00	1.00	7.00
7	3.00	5.00	7.00	1.00
8	3.00	7.00	4.00	2.00
9	5.00	1.00	4.00	7.00
10	5.00	3.00	7.00	4.00
11	5.00	5.00	1.00	2.00
12	5.00	7.00	2.00	1.00
13	7.00	1.00	7.00	2.00
14	7.00	3.00	4.00	1.00
15	7.00	5.00	2.00	7.00
16	7.00	7.00	1.00	4.00

Table 3
Composition of the validation samples

Samples	Concentration (mgl^{-1})			
	Maltol	Ethyl maltol	Vanillin	Ethyl vanillin
1	1.20	1.20	1.20	1.20
2	1.20	3.50	3.00	3.00
3	1.20	4.50	5.00	5.00
4	1.20	6.50	6.50	6.50
5	2.50	1.20	3.00	5.00
6	2.50	3.50	1.20	6.50
7	2.50	4.50	6.50	1.20
8	2.50	6.50	5.00	3.00
9	4.50	1.20	5.00	6.50
10	4.50	3.50	6.50	5.00
11	4.50	4.50	1.20	3.00
12	4.50	6.50	3.00	1.20
13	6.50	1.20	6.50	3.00
14	6.50	3.50	5.00	1.20
15	6.50	4.50	3.00	6.50
16	6.50	6.50	1.20	5.00

2.6. Interferences

Various possible interfering substances, such as sucrose, glucose, citric acid, synthetic colorants and metal

ions, all of which are commonly present with the flavor enhancers in commercial food samples, were tested under the same experimental conditions. The effect of interference with different concentrations on the absorbance of a solution containing 4 mgl^{-1} of each analyte was studied. It was generally considered to interfere with the analysis when its presence produced a variation in the absorbance of the sample larger than 5%. It was found that sucrose, glucose and citric acid did not interfere at a 500:1 interferent-to-analyte concentration ratio. Synthetic colorants, such as sunset yellow, tartrazine, amaranth and ponceau 4R, produced interferences at nearly 10 times concentration level to flavor enhancers. Na^+, K^+, Ca^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, Cd^{2+} and Cu^{2+} did not interfere or produced only small contributions even at a 100:1 inerferent-to-analyte ratio. It was found that Fe^{3+} produced serious interference in the determination. However, the analytes were extracted with anhydrous ethanol in real sample analysis, and Fe^{3+} is not extracted, so no interference occurs.

2.7. Application to food samples

Several commercial food samples were purchased from the local market in Nanchang city. The food sample was ground to a fine powder with a mortar and pestle. 30.0 g of this powder and 50 ml of anhydrous ethanol were placed into a 100 ml Erlenmeyer flask (with a screw cap), and shaken by a laboratory shaker for 2 h, this mixture was then transferred to a 10 ml centrifuge tube, and centrifuged at 3000 rpm for 5 min. The clear part of the solution in the tube was used for analysis. Suitable amount of this sample was transferred into a 25 ml flask, added 5.0 ml of 95% ethanol, then the solution was analyzed by the procedure as described in Section 2.3.

3. Results and discussion

3.1. Chemometrics

Multivariate calibration approaches are important applications in spectrophotometric multicomponent analysis. Here **A** and **C** represent the matrices of absorbance and concentration of a set of standard solutions, respectively, and assuming that m standard solution mixtures containing n kinds of component are prepared according to the orthogonal array design (Lan et al., 1994), and then their absorbances are measured at l wavelengths, the following equation can be obtained:

$$\mathbf{A}_{m\times l} = \mathbf{C}_{m\times n}\mathbf{K}_{n\times l}, \tag{3}$$

where **K** is the coefficient matrix. According to this equation, it is possible to determine the components individually with the use of suitable chemometric methods.

CLS (Schmidt Peter & Glombitza Bernhard, 1995) is a very common multivariate calibration method and has been used for quantitative spectral analysis. This method has generally presumed that there is a linear relationship between response signals and component concentrations. In addition, this method has a calibration step where the relationship between the spectra and component concentrations is estimated from a set of standard samples. This step is followed by prediction in which the results of the calibration are used to estimate the component concentrations from the spectrum of 'unknown' samples. A major disadvantage of CLS is that all interfering chemical components in the spectral region of interest need to be known and be included in the calibration models.

PCR (Jolliffe, 1986) and PLS (Markus, 1996) are factor analysis based multivariate statistical tools, which have many of the full-spectrum advantages of the CLS method, and have been successfully applied to the analysis of multicomponent mixtures. Like the CLS method, PCR and PLS need a calibration step where the models for the spectra and the component concentrations are deduced from a set of standards, followed by a prediction step in which the concentrations of the unknown are estimated from the sample spectrum. Both of these methods involve spectral decomposition. The PCR decomposition is based entirely on spectral variations without regard for the component concentrations, and in PLS, the spectral decomposition is weighted to the concentration.

Artificial neural networks (ANN) are widely used to solve some analytical problems, the most popular is the multilayer feed-forward net with the back-propagation (BP-ANN) learning algorithm (Zupan & Gasteiger, 1991). Recently, a potential alternative approach, ANN-radial basis function (RBF), has been developed, which offers some advantages of robustness and sensitivity to noisy data as comparing with ANN-BP. The basic theory for RBF-ANN and application to chemical problems are found in the literature (Walczak & Massart, 1996; Pulido, Ruisanchez, & Ruis, 1999).

The structure of RBF is comprised of three node layers of a pass-through input layer, a hidden layer and an output layer. Each neuron of the hidden layer represents a kernel or basis function, its dimensionality being the same as the input data. For example, if the dimensionality of the input data equals 20 (i.e., number of input nodes, $i=20$), the RBF is 20-dimensional. RBF networks generally use a Gaussian function to account for the non-linearity of the hidden layer processing elements and the Gaussian function responds only to a small region of the input space where the Gaussian is centered. The key to a successful implementation of these networks is to find suitable centers for such a Gaussian function, which is characterized by two parameters, i.e., center (c_j), and peak width (σ_j). The

output from the jth Gaussian neuron for an input object x_i can be calculated by the following equation:

$$\text{out}_j = \Phi_j(\|x_i - c_j\|) = \exp\left(\frac{-\|x_i - c_j\|^2}{(\sigma_j)^2}\right), \quad (4)$$

where $\|x_i - c_j\|$ is the Euclidean distance between x_i, and c_j, and σ_j determines the portion of the input space where the jth RBF will have a non-significant zero response. This RBF hidden layer is fully connected to the output layer by the size of the weight coefficients, w_{kj}, and the response of each output node is calculated by a linear function of its input (which includes the bias w_{kj}), that is, the output of the hidden layer (out_k). The relationship between value out_k and the input variables x_i can be represented by

$$\text{out}_k = w_{k0} + \sum_j w_{kj}\Phi_j(\|x_i - c_j\|). \quad (5)$$

The weights w_{kj} are adjusted to minimize the mean square error of the net output. There are two sets of parameters (the centers and the widths) in the hidden layer and a set of weights in output layer are adjusted, and the adjustment for output layer is simple, so the RBF neural network has a guaranteed learning procedure for convergence.

3.2. Absorption spectra

The absorption spectra over the 200–350 nm of the four individual flavor enhancer solutions are shown in Fig. 1. It can be seen that the spectra of these compounds overlapped seriously, and quantitative estimations cannot be carried out successfully by conventional calibration methods. Both MAL and EMA have two peaks, 213 and 273 nm for MAL and 213 and 274 nm for EMA, while both VAN and EVA have three peaks, 229, 278 and 308 nm for VAN and 230, 279 and 309 nm for EVA. The absorbance data from 200 to 350 nm was used for calibration in this work, because most quantitative information can be extracted from this region.

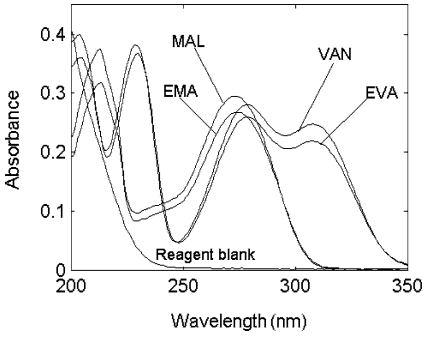

Fig. 1. Absorption spectra of maltol (MAL), ethyl maltol (EMA), vanillin (VAN), ethyl vanillin (EVA) and the reagent blank. Concentration: 4.0 mgl^{-1}.

3.3. Effect of pH on the absorbance of the compounds

The effect of pH on the absorbance of these compounds was investigated in this work. A set of Britton–Robinson buffer solutions with different pH in the range of 1.81–11.2 were prepared, and the spectra of each compound in these buffers were measured from 200 to 400 nm (Fig. 2). It can be seen that constant absorbances and well-defined spectra of each flavor enhancer can be observed in the pH range of 1.81–6.00. In this work, pH 2.87 is chosen as the optimum pH for experiments. The absorbances of VAN and EVA started to increase with increasing pH for pH > 6 until finally the peak maxima of these two compounds shifted to 347 nm. Similarly, as the absorbances of MAL and EMA increased with increasing pH for pH > 8, the peak wavelengths of these two compounds shifted to 221 and 319 nm, respectively. The reason for all these variations probably involves the hydroxyl groups in the molecular structures of the analytes. Their degree of protonation would change with the change in pH. When pH > 6, VAN and EVA exist as phenyl salt i.e., the OH is deprotonated to some extent, while in the pH range of 2.0–6.0, the OH is protonated giving rise to the neutral form of the molecule. Similarly, when pH > 8, MAL and EMA exist as the phenyl salts, but are protonated in the pH range of 2.0–8.0.

3.4. Comparison of different chemometrics methods

Sixteen synthetic mixtures containing the four flavor enhancers were prepared for validation (Table 3), and the RPE (%) and the recovery (%) found by each chemometrics method were listed in Table 4. It can be seen that RBF-ANN gives the best results on the basis of %RPE and %recoveries, presumably because this ANN method is particularly well suited for modeling non-linear and complex systems. Both CLS and DCLS perform poorer, they give higher RPE_T, and the recoveries are also not satisfactory. Because these two methods generally cannot account for any non-linearity between components, they do not model well complex analytical systems. PLS, PCR, DPLS, and DPCR give better results, their RPE_T are less than 10% and give acceptable recoveries. When the derivative spectral matrix was analysed (DCLS, DPCR and DPLS), it was found that the prediction errors (RPE_T) did not improve. From Table 4 it can also be seen that most methods, except for CLS and DCLS, give acceptable RPE_S (2.7–10%) and recoveries (88–112%) for these four compounds. It can also be seen that both PLS and PCR gave similar results, for they are methods based on factor analysis and are suitable for the analysis of many complex systems. It is important to select suitable number of factors for the PCR and PLS models, and Figs. 3 and 4 show the relationship between the RPE_T and the

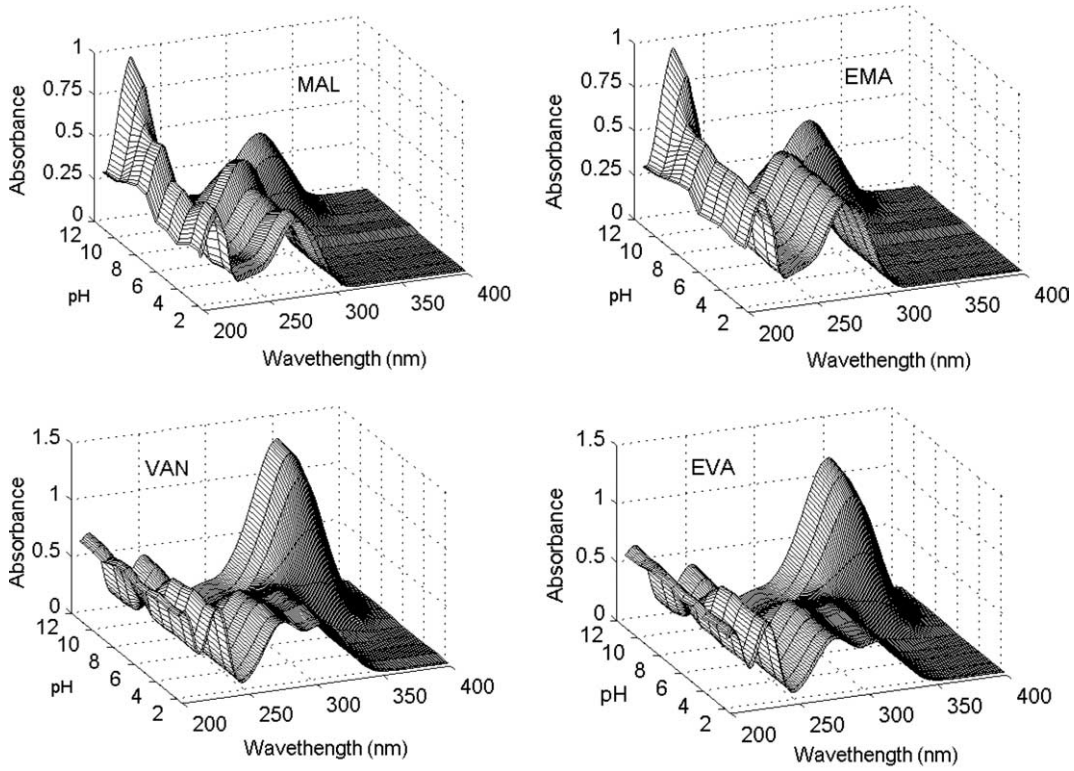

Fig. 2. Absorbance of maltol (MAL), ethyl maltol (EMA), vanillin (VAN) and ethyl vanillin (EVA) with pH.

Table 4
Prediction results for each compound from the validation samples by different chemometrics methods

Methods	RPE_S (%) and recovery (%) for compounds				RPE_T (%)
	Maltol	Ethyl maltol	Vanillin	Ethyl vanillin	
CLS	25.0 (78)[a]	26.6 (136)	3.4 (100)	4.8 (98)	18.3
DCLS[b]	26.9 (86)	30.7 (138)	6.7 (105)	6.8 (98)	20.7
PCR[c]	8.8 (102)	9.9 (108)	5.1 (103)	6.1 (99)	7.7
DPCR	8.9 (112)	9.4 (95)	8.4 (111)	9.8 (89)	9.1
PLS	9.4 (99)	10.4 (110)	4.3 (99)	5.3 (97)	7.7
DPLS	8.9 (104)	9.3 (95)	8.9 (110)	10.1 (88)	9.4
RBF-ANN[d]	5.1 (104)	5.3 (99)	2.7 (101)	4.3 (97)	4.4

[a] The values in parentheses correspond to the mean recoveries (%). Recovery $(\%) = 100 \times \sum_{i=1}^{n}(c_{i(\mathrm{pred})}/c_{i(\mathrm{real})})/n$, where n is the number of samples.

[b] "D" indicates the first-derivative pre-treatment approach.

[c] The number of factors selected were: 8 for PCR and DPCR, 9 for PLS, and 7 for DPLS.

[d] The parameters of radial neurons, sum-squared error goal and spread of radial basis function for were 14, 0.02 and 80, respectively.

number of factors used for calibration method. Thus, with reference to these graphs, the number of factors selected for PCR, DPCR, PLS, and DPLS were 8, 8, 9 and 7, respectively.

The GC–MS technique (Adahchour et al., 1999) for the determination of VAN and MAL in butter was based on solid-phase extraction, and limits of detection of 2–10 pg were reported. The LC technique (Portela et al., 1996) coupled with the oxidative amperometric detection and a glassy carbon electrode was applied for the determination of MAL in cake samples. The linear response was observed to be from 3 to 40 ng. The sensitivity of these two methods was high, but complex previous separation was generally needed, which was time-consuming. Square-wave voltammetry (SWV) has been used to determine the VAN in dehydrated pudding powder dissolved in ethyl acetate (Agui et al., 1999). The linear range was 1.5–106 mgl^{-1} and the limit of detection was 0.6 mgl^{-1}. UV–Vis spectrometric procedures are commonly applied in many cases due to their good reliability, simplicity and reproducibility. The procedure for spectrophotometric determination of EMA with the reagent ferric chloride was reported with the linear range of 1.5–15 mgl^{-1} (Cheng et al.,

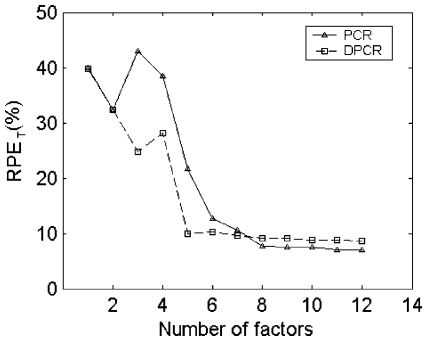

Fig. 3. Relationship of RPE_T and the number of factors for PCR and DPCR modelling.

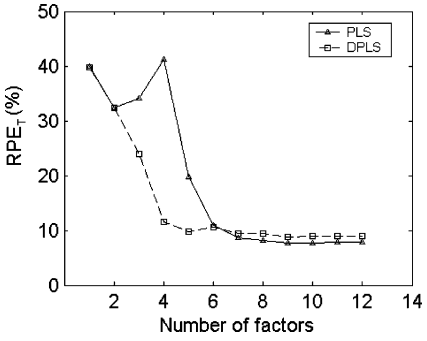

Fig. 4. Relationship of RPE_T and the number of factors for PLS and DPLS modelling.

2000). It can be seen that, generally, the linear ranges and limit of detection for voltammetry and UV–Vis spectrophotometry are similar to the proposed method (Table 1). Further, only one or two compounds can be determined simultaneously by the use of the conventional data interpretation methods because the spectra measured are seriously overlapped. In this work, chemometrics approaches were applied to resolve the overlapping spectra, and determine MAL, EMA, VAN and EVA simultaneously. The linear ranges of the four compounds were 1.0–20.0 mgl^{-1} and the limit of detections for these four flavor enhancers are in the range of 0.38–0.56 mgl^{-1}, which is an improvement of at least a factor of two on the number of analytes estimated together. This indicates significant analytical cost savings.

3.5. Results for real samples

From the discussion in Section 3.4, it was shown that PLS and RBF-ANN are the two well performing methods for prediction. Therefore, these methods were applied for analysis of real food samples and the results are summarized in Table 5. As can be seen from the results, VAN was the single, frequently used flavor enhancer in food products in China. EMA and VAN were sometimes mixed in foods, and the amounts of VAN are much higher than that of EMA in Preserved Fruit and Milk Candy samples. Also, Table 5 lists the recoveries obtained from the standard additions to each sample. As can been seen, overall, PLS and RBF-ANN give similar mean %recoveries and are practically indistinguishable in performance. In general, most are in the range from 82% to 117%, except for one poor of result of 79%.

Table 5
Results of analysis for flavor enhancers in real food samples

Samples[a]	Found in samples (mgg^{-1})				Added (mgg^{-1})				Found for addition (mgg^{-1})				Recovery (%)			
	MAL	EMA	VAN	EVA	MAL	EMA	VAN	EVA	MAL	EMA	VAN	EVA	MAL	EMA	VAN	EVA
Method of PLS[b]																
1	ND[c]	ND	0.37	ND	0.28	0.28	0.28	0.28	0.29	0.30	0.66	0.26	104	109	104	95
2	ND	ND	0.29	ND	0.28	0.28	0.28	0.28	0.31	0.28	0.54	0.24	111	103	92	85
3	ND	ND	0.45	ND	0.28	0.28	0.28	0.28	0.26	0.23	0.77	0.30	93	82	115	108
4	ND	ND	0.33	ND	0.14	0.14	0.37	0.30	0.10	0.16	0.74	0.38	79	114	112	103
5	ND	0.09	0.38	ND	0.14	0.14	0.37	0.30	0.12	0.24	0.75	0.41	89	106	98	111
6	ND	0.06	0.31	ND	0.14	0.14	0.37	0.30	0.15	0.22	0.63	0.35	109	113	87	94
Method of RBF-ANN[d]																
1	ND	ND	0.34	ND	0.28	0.28	0.28	0.28	0.27	0.29	0.68	0.27	98	104	113	96
2	ND	ND	0.26	ND	0.28	0.28	0.28	0.28	0.30	0.31	0.54	0.30	108	113	100	106
3	ND	ND	0.44	ND	0.28	0.28	0.28	0.28	0.32	0.27	0.75	0.26	115	97	112	92
4	ND	ND	0.32	ND	0.14	0.14	0.30	0.37	0.13	0.15	0.73	0.43	95	109	109	117
5	ND	0.08	0.41	ND	0.14	0.14	0.37	0.37	0.12	0.23	0.78	0.39	86	107	100	105
6	ND	0.07	0.29	ND	0.14	0.14	0.37	0.37	0.15	0.23	0.68	0.41	114	111	105	111

[a] Samples: (1) Conserved plum. Guangdong Chaoan Food Production Co. Ltd. (2) Egg roll. purchased from Rainbow supermarket, Nanchang city. (3). Chocolate. Shanghai Golden Monkey Production Co. Ltd. (4) Caramel candy. Shanghai Perfetti Candy Production Co. Ltd. (5). Preserved fruit. Guangdong Funhuang Food Production Co. Ltd. (6) Milk candy. Shanghai Golden Monkey Food Production Co. Ltd.

[b] Nine factors were modelled.

[c] Not detected.

[d] Parameters used were as in Table 4.

4. Conclusions

In this paper, a rapid and simple spectrophotometric procedure was developed for the simultaneous determination of four flavor enhancers, MAL, EMA, VAN and EVA, with the aid of chemometrics approaches. The prediction of the concentrations of the compounds was facilitated by the use of an orthogonal design to build a calibration dataset, which was then applied for the building of calibration models with seven different chemometrics methods for the testing of a validation data set constructed from synthetic solutions of the four compounds. The analytical performance of these chemometric methods was characterized by %RPE and %recoveries. The results obtained by the application of the different chemometrics approaches showed that RBF-ANN was the preferred method on the basis of %RPE and %recoveries. PCR, PLS, DPCR and DPLS also gave satisfactory results, while CLS and DCLS performed poorly. Derivative spectral matrices did not improve the prediction errors (%RPE_T). Experimental data of real samples was processed by PLS and RBF-ANN with satisfactory results, which were supported by the recoveries of standard additions.

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