Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 431–438
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Analytical Methods

Simultaneous kinetic-spectrophotometric determination of maltol and ethyl maltol in food samples by using chemometrics

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Received 1 September 2007; received in revised form 2 December 2007; accepted 15 December 2007

Abstract

A fast and accurate procedure has been researched and developed for the simultaneous determination of maltol and ethyl maltol, based on their reaction with iron(III) in the presence of *o*-phenanthroline in sulfuric acid medium. This reaction was the basis for an indirect kinetic spectrophotometric method, which followed the development of the pink ferroin product ($\lambda_{max} = 524$ nm). The kinetic data were collected in the 370–900 nm range over 0–30 s. The optimized method indicates that individual analytes followed Beer's law in the concentration range of 4.0–76.0 mg L^{-1} for both maltol and ethyl maltol. The LOD values of 1.6 mg L^{-1} for maltol and 1.4 mg L^{-1} for ethyl maltol agree well with those obtained by the alternative high performance liquid chromatography with ultraviolet detection (HPLC-UV). Three chemometrics methods, principal component regression (PCR), partial least squares (PLS) and principal component analysis–radial basis function–artificial neural networks (PC–RBF–ANN), were used to resolve the measured data with small kinetic differences between the two analytes as reflected by the development of the pink ferroin product. All three performed satisfactorily in the case of the synthetic verification samples, and in their application for the prediction of the analytes in several food products. The figures of merit for the analytes based on the multivariate models agreed well with those from the alternative HPLC-UV method involving the same samples.

Keywords: Kinetic determination; Maltol; Ethyl maltol; Chemometrics; Artificial neural networks

1. Introduction

Maltol (3-hydroxy-2-methyl-4-pyrone), a naturally occurring substance, has been marketed as a food flavor enhancing agent (Ellis, 1972). Its synthetic homologue, ethyl maltol, which is approximately six times more effective than maltol (Rennhard, 1971), has been available since 1967. These compounds are often found as flavor enhancers in many foods such as coffee, soybeans, cereals, breads, malt beverages, and chocolate milk (Heath, 1978; Hui, 1991; LeBlanc & Akers, 1989). Maltol is tasteless at the recommended application doses, rather it modifies or enhances the flavors of the foods and beverages to which it is added. However, Gralla, Stebbins, Coleman, and Delahunt (1969) has reported some concerns regarding the biological health and safety of ethyl maltol. Therefore, the determination of these two substances in foods is clearly important, and methods, which can analyze quantitatively the two compounds simultaneously, would be of particular advantage.

Some high performance liquid chromatography with ultraviolet detection (HPLC-UV) and mass spectrometry (MS) have been reported for the determination of either maltol (Ferreira, Jarauta, Lopez, & Cacho, 2003) or ethyl maltol (Liu, Wang, Yang, & Yin, 2006; Wang, Liu, Yang, Tian, & Kou, 2006) in foods. However, the above noted techniques employ expensive instruments and/or materials, and high purity solvents. HPLC-UV methods require

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doi:10.1016/j.foodchem.2007.12.036

suitable compounds for internal standards, which add to the cost and complexity of the analytical procedure. Moreover, reports on the simultaneous determination of the two compounds in such samples are a few (Ni, Zhang, & Kokot, 2005; Peng, Ma, & Di, 2005).

Recent publications, which described the differential kinetic spectrophotometric methods coupled with chemometrics data of interpretation, have demonstrated the success of this approach for simultaneous analysis of similar substances (Crouch, Coello, Maspoch, & Porcel, 2000; Ni, Huang, & Kokot, 2004; Ni & Wang, 2007). In particular, these spectrophotometric methods can be increasingly applied for the simultaneous determination of two homologues in food and pharmaceutical samples (Abbaspour & Mirzajani, 2006; Blanco, Coello, Iturriaga, Maspoch, & Porcel, 1999; Chamsaz, Safavi, & Fadaee, 2007; Ni, Wang, & Kokot, 2007), because it is difficult for them to be quantitatively analyzed by traditional spectrophotometry because of their overlapping UV spectra. The principles and applications of the differential kinetic methods have been reviewed (Crouch, 1993; Quencer & Crouch, 1993). Essentially, in these methods similar analyte species react with a common reagent, and differences in the reaction kinetics are used to distinguish the components without any physical separation. Chemometrics techniques are used for the processing of kinetic data. Such an approach does not require a detailed kinetic model. This is a major advantage over the conventional techniques for processing kinetic data, because such techniques rely on an accurate kinetic model of the chemical system to obtain the order of reaction and rate constants.

In this work, a differential kinetic-spectrophotometric method has been investigated for the simultaneous analysis of the two analytes, maltol and ethyl maltol. The method relies on the different kinetic responses of maltol and ethyl maltol, which react with iron(III) in the presence of *o*-phenanthroline in the sulfuric acid medium. Calibration models were built from the kinetic data derived from the analyte mixtures, and with the aid of multivariate methods of analysis such as principal component regression (PCR), partial least squares (PLS) and principal component analysis–radial basis function–artificial neural networks (PC–RBF–ANN). These models were verified, compared and applied for prediction of analytes in real samples.

2. Methodology

2.1. Kinetic models

Consider two analytes, A and B, which react with a common reagent, R, to give the absorbing products, P_A and P_B, according to the following reactions:

$$A + R \rightarrow P_A \quad (1)$$

$$B + R \rightarrow P_B \quad (2)$$

Assume that the two reactions involved, follow first or pseudo-first order kinetics with respect to the analyte. Thus, the rate equations for A and B are

$$-\frac{dc_A}{dt} = k_A c_A \quad (3)$$

$$-\frac{dc_B}{dt} = k_B c_B \quad (4)$$

where c_A and c_B are the concentrations of analytes, A and B, at time t, and k_A and k_B are the corresponding rate constants.

Integration of Eqs. (3) and (4) yields

$$c_A = c_{A,0}\exp(-k_A t) \quad (5)$$

$$c_B = c_{B,0}\exp(-k_B t) \quad (6)$$

where $c_{A,0}$ and $c_{B,0}$ are the initial concentrations of analytes, A and B, respectively.

Given the stoichiometry between the analyte and product, the concentrations of P_A and P_B at time, t, can be represented as follows:

$$c_{P_A} = c_{A,0}[1 - \exp(-k_A t)] \quad (7)$$

$$c_{P_B} = c_{B,0}[1 - \exp(-k_B t)] \quad (8)$$

where c_{P_A} and c_{P_B} represent the concentrations of P_A and P_B at time, t, during the reaction process, respectively.

When the two analytes behave independently and the spectral absorbances of their products, i.e., P_A and P_B, are additive, the absorbance of a mixture of A and B may be written as

$$\begin{aligned} A &= A_{P_A} + A_{P_B} = \varepsilon_{P_A} b c_{P_A} + \varepsilon_{P_B} b c_{P_B} \\ &= c_{A,0}\varepsilon_{P_A} b[1 - \exp(-k_A t)] + c_{B,0}\varepsilon_{P_B} b[1 - \exp(-k_B t)] \\ &= K_A c_{A,0} + K_B c_{B,0} \end{aligned} \quad (9)$$

where ε_{P_A} and ε_{P_B} are molar absorptivities of P_A and P_B, respectively; K_A and K_B are coefficients of proportionality for components, A and B, at time t, respectively, and b is the cell length.

For m standard samples, the absorbance data of kinetic systems being monitored at time, s, can be expressed in matrix form as

$$\boldsymbol{A}_{m\times s} = \boldsymbol{C}_{m\times 2}\boldsymbol{K}_{2\times s} \quad (10)$$

According to this equation, it is possible to determine the components (in this work – maltol and ethyl maltol) by a suitable chemometrics method. Thus, in this study, the kinetic data were collected from experiments and then processed by PCR, PLS and PC–RBF–ANN.

2.2. Chemometrics methods

2.2.1. Principal component regression (PCR) and partial least squares (PLS)

Principal component regression (PCR) and partial least squares (PLS) are two well-known full spectrum multivariate calibration methods (Martens & Naes, 2001). These factor analysis based methods can overcome signal over-

lapping (Malinowski, 2002), and they have been discussed extensively in the literature (David & Thomas, 1988; Geladi & Kowalski, 1986; Lorber, Wangen, & Kowalski, 1987; Ni et al., 2007; Wentzell & Montono, 2003). With PCR, the data decomposition is performed using only the ***X***-matrix, i.e., the signal information, while PLS employs the ***Y***-matrix, i.e., the concentration data, as well as the ***X***.

2.2.2. Principal component analysis–radial basis function–artificial neural network (PC–RBF–ANN)

Artificial neural network (ANN) is a model, which is based on a simplified concept of the functioning of the brain. The model involves a number of nodes, called processing elements or neurons, which are interconnected in a net-like structure (Rosenblatt, 1958). A network consists of three node layers: an input, a hidden and an output layer. The input nodes only distribute the input vectors to the hidden layer, which contains a transfer function, and the output layer. Such models are readily applied to linear and non-linear problems (Bishop, 1995; Haykin, 1998).

In this work, the data matrix was initially submitted to PCA for data pretreatment. Scores from the significant new latent variables were then processed by RBF–ANN in the hidden layer. This layer consisted of a number of RBF neurons (n_h) and bias (b_k). Each neuron employed a radial basis function as the non-linear transfer function to operate on the scores data obtained by PCA. A common RBF is the Gaussian function, $o_j(x)$, which is characterized by the centre (c_j) and the width (r_j)

$$o_j(x) = \exp[-(||x_i - c_j||/r_j)^2] \tag{11}$$

In the iterative calculation, spread is the most important parameter of RBF–ANN networks, and it controls the size of the width (r_j). The larger the spread, the flatter and smoother will be the Gaussian function approximation. If the spread is too large, then many neurons will be required to fit a fast changing function; if the spread is too small, then many neurons are needed to obtain a smooth function, and the network may not generalize well.

3. Experimental

3.1. Chemicals and reagents

All chemicals used were of analytical reagent grade, and all solutions were prepared with doubly distilled water. Stock solutions of maltol (1 g L^{-1}) and ethyl maltol (1 g L^{-1}) were prepared from suitable weight aliquots, and dissolved in water in a 100 mL volume flask; the solution was then diluted to the mark with water and mixed well. A sulfuric acid solution (1 mol L^{-1}), an iron(III) solution (0.04 mol L^{-1}) and an *o*-phenanthroline solution (0.02 mol L^{-1}) were prepared by taking suitable weight aliquots of the reagents and dissolving them in water.

3.2. Instrumentation

Kinetic and spectral data were obtained from measurements on an Agilient 8453 UV–visible spectrophotometer with a 10 mm fused-silica cuvette. A Model ZC-10 thermostat (Ningbo Tianheng Instruments Factory, China) was used to maintain the reaction temperature at 16 °C. The pH was measured with a Model SA-720 pH meter (Orion). The sample solutions were subjected to a short sonication (SK1200H, Kudos Ultrasonic Instrument Co. Ltd., Shanghai), and all solution volumes of less than 1 mL were delivered with micropipettes (Finnpipette, Labsystems, Finland).

HPLC measurements were carried out with an Agilent 1100 Series HPLC-DAD system including a vacuum degasser, quaternary pump, autosampler, and an injector with a 100 μL loop. An Agilent ZORBAX Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm) was used with an Agilent Zorbax high pressure reliance cartridge guard-column (C18, 12.5 mm × 4.6 mm, 5 μm), and a variable wavelength UV–visible detector measuring at 274 nm. All chromatograms were run at room temperature using a 40:60 mixture of 0.2% $KH_2PO_4 \cdot H_3PO_4$ buffer solution and methanol. Other experimental parameters were: flow rate, 1 mL min^{-1}; retention times, 3.6 min for maltol and 4.6 min for ethyl maltol.

3.3. Data processing

Data processing was performed by a Pentium IV computer. The PCR, PLS and PC–RBF–ANN algorithms were written in MATLAB (Mathworks version 6.5) by the authors. The spectrophotometric absorbance versus time data were autoscaled prior to submission to the appropriate algorithms. The optimum number of PCs for PCR, PLS and PC–RBF–ANN models was determined with the use of cross-validation procedure (leave-one-out method). To minimize the prediction error, the sum of squares (PRESS) between estimated and added concentrations was computed

$$\text{PRESS} = \sum_{i=1}^{n} \left(c_{ij(\text{estimated})} - c_{ij(\text{added})}\right)^2 \tag{12}$$

where $c_{ij(\text{estimated})}$ and $c_{ij(\text{added})}$ are the estimated and added analyte concentrations of *j*th component in *i*th mixtures in the calibration set, respectively.

This is a well-known parameter, which can be used to indicate how well the three chemometrics models fit the concentration data. One criterion for the selection of the optimum number of factors is the number, which coincides with the minimum PRESS value. However, the PRESS calculation is based on a finite number of samples and, therefore, it is subject to error. Hence, such use of PRESS can lead to overfitting. A better criterion involves the comparison of the minimum PRESS – factor number combination with such other combinations calculated progressively. Such comparisons are generally facilitated by the calculation of

the well known *F*-ratios. Haaland and Thomas (1988) have suggested that an *F*-ratio of 0.75 is a satisfactory minimum threshold to select the number of significant factors, and this criterion was applied in this work.

The performance of the calibration models was verified with the analysis of a data matrix from synthetic binary mixtures. The parameters chosen to evaluate the prediction performance were: % recovery for individual compounds, relative prediction error – % RPE_S for individual compounds, and % RPE_T for all compounds, Eqs. (13)–(15) (Otto & Wegscheider, 1985)

$$\%\ \text{Recovery} = 100 \times \sum_{i=1}^{n} \left(c_{ij(\text{estimated})}/c_{ij(\text{added})}\right)/n \tag{13}$$

$$\%\ \text{RPE}_S = 100 \times \left[\frac{\sum_{i=1}^{n}\left(c_{ij(\text{estimated})} - c_{ij(\text{added})}\right)^2}{\sum_{i=1}^{n}\left(c_{ij(\text{estimated})}\right)^2}\right]^{0.5} \tag{14}$$

$$\%\ \text{RPE}_T = 100 \times \left[\frac{\sum_{i=1}^{n}\sum_{j=1}^{m}\left(c_{ij(\text{estimated})} - c_{ij(\text{added})}\right)^2}{\sum_{i=1}^{n}\sum_{j=1}^{m}\left(c_{ij(\text{added})}\right)^2}\right]^{0.5} \tag{15}$$

where $c_{ij(\text{added})}$ indicates the actual concentration of *j*th component in *i*th mixtures in the verification data set, $c_{ij(\text{estimated})}$ is its estimation by the calibration models, and *n* is the number of samples.

3.4. General procedure

A volume of *x* (<0.4) mL of the analyte solution was placed into a 1 cm cuvette, and 0.03 mL of 0.02 mol L^{-1} *o*-phenanthroline solution, together with 0.03 mL of 1 mol L^{-1} of sulfuric acid were then added, followed by (2.34 − *x*) mL water to give a volume of 2.4 mL. The cell was shaken, and allowed to stand for 2 min in the temperature controlled holder of the spectrophotometer (16 °C) before the absorbance was set to zero. The cell was removed, and the final 0.1 mL of 0.04 mol L^{-1} of iron(III) was rapidly added to give 2.5 mL. Kinetic curves were followed simultaneously from 370 to 900 nm, every 1 nm, at 0.5 s intervals during 30 s.

3.5. Procedure for the determination of maltol and ethyl maltol in food samples

Several commercial food samples were purchased from the market in Nanchang city. A solid food sample (~100 g), such as biscuit and jelly, was ground to a fine powder. And a liquid food sample (~100 mL), such as beverage and julep, was filtered. Subsequently, this powder (75.0 g) or the filtrate (75 mL) with 150 mL of chloroform was placed into a 250 mL Erlenmeyer flask (with a screw cap), and then shaken for 2.5 h. A suitable aliquot of this mixture was then transferred to a 10 mL centrifuge tube, and centrifuged at 4000 rpm for 10 min. The clear portion of the mixture in the tube was used for analysis. An appropriate amount of this sample and 5.0 mL of chloroform were added to a 25 mL flask, then diluted to the mark with water and mixed thoroughly. Such solution was used for analysis by the general procedure described above (Section 3.4).

3.6. HPLC procedure

Twenty microlitres of each sample (solutions from Section 3.5) were injected, and the concentrations were calculated on the basis of the peak area ratios.

4. Results and discussion

4.1. Spectra behaviour

Absorption spectra of maltol and ethyl maltol in aqueous solution (Fig. 1A) indicated that they overlapped

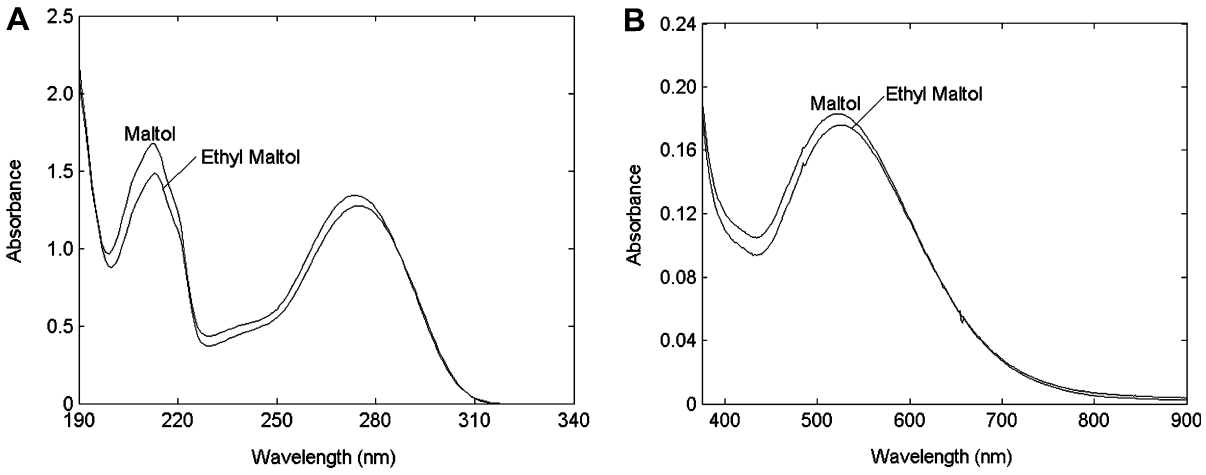

Fig. 1. (A) UV–visible spectra of maltol (20.0 mg L^{-1}) and ethyl maltol (20.0 mg L^{-1}) in aqueous solution. (B) UV–visible absorption spectra of maltol (20.0 mg L^{-1}) and ethyl maltol (20.0 mg L^{-1}) at $t = 30$ s. Other experimental conditions are: T=16 °C, $c_{\text{iron(III)}} = 1.6 \times 10^{-3}$ mol L^{-1}, $c_{o\text{-phenanthroline}} = 2.4 \times 10^{-4}$ mol L^{-1}, $c_{H_2SO_4} = 0.012$ mol L^{-1}.

strongly, and had two common peaks at 213 and 273 nm. This ruled out prediction by conventional spectral analysis, and led to the kinetic studies and the application of multivariate calibrations.

4.2. Reaction kinetics

According to previous studies (Ghasemi, Seifi, Sharifi, Ghorbani, & Amini, 2004; Ghasemi, Seraji, Noroozi, Hashemi, & Jabbari, 2004; Martinovic, Kukoc-Modun, & Radic, 2007), maltol or ethyl maltol reduced iron(III) in the presence of *o*-phenanthroline in the sulfuric acid medium to give the pink ferroin (Fig. 2). Spectra of the coloured ferroin, obtained from reactions under the experimental conditions described (Section 3.4), were measured in the range of 370–900 nm at a reaction time of 30 s (Fig. 1B). These spectra included an absorption band at 524 nm, which corresponded to the reaction product, ferroin. The absorbance versus time kinetics measured over 30 s together with the spectra (Fig. 1B), indicate that both analytes yield the same reaction product, ferroin, but at a different reaction rates. The rate constants for the reactions involving maltol and ethyl maltol, which were estimated by fitting the kinetic data, obtained from single component samples, to the equation of $A = a_0 - a_1\exp(-kt)$ (Draper & Smith, 1981), are 0.0471 and 0.0394, respectively. Clearly, there are only small differences between the reaction rates of maltol and ethyl maltol. Thus, it is difficult to use classical differential kinetic methods, such as the logarithmic extrapolation and the proportional equation, to resolve the mixtures. Therefore, the application of PCR, PLS and PC–RBF–ANN modeling was investigated.

4.3. Optimization of the reaction conditions and univariate calibration

The effects of the concentrations of iron(III), *o*-phenanthroline, sulfuric acid and temperature on the determination of maltol or ethyl maltol were optimized. The results showed that the optimized experimental conditions were 1.6×10^{-3} mol L^{-1} for iron(III), 2.4×10^{-4} mol L^{-1} for *o*-phenanthroline, 0.012 mol L^{-1} for sulfuric acid and 16 °C for temperature. Under these conditions, several calibration samples with different concentrations of maltol or ethyl maltol were analyzed, and the absorbance response was measured as a function of time at 524 nm for each food flavor enhancing agent (Fig. 3). Parameters of the linear models for these data showed that for both compounds, there was a good linear correlation between the measured absorbance (A) and concentration (c). The calibration equations were $A = 8.2 \times 10^{-3}c + 21.3 \times 10^{-3}$ ($n = 10$, $r = 0.9998$) for maltol, and $A = 7.5 \times 10^{-3}c + 27.8 \times 10^{-3}$

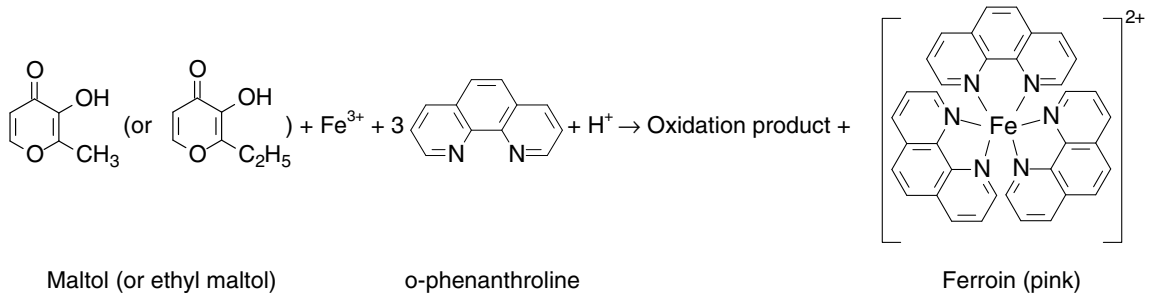

Fig. 2. Oxidation reaction of maltol or ethyl maltol by iron(III) in the presence of *o*-phenanthroline in sulfuric acid medium.

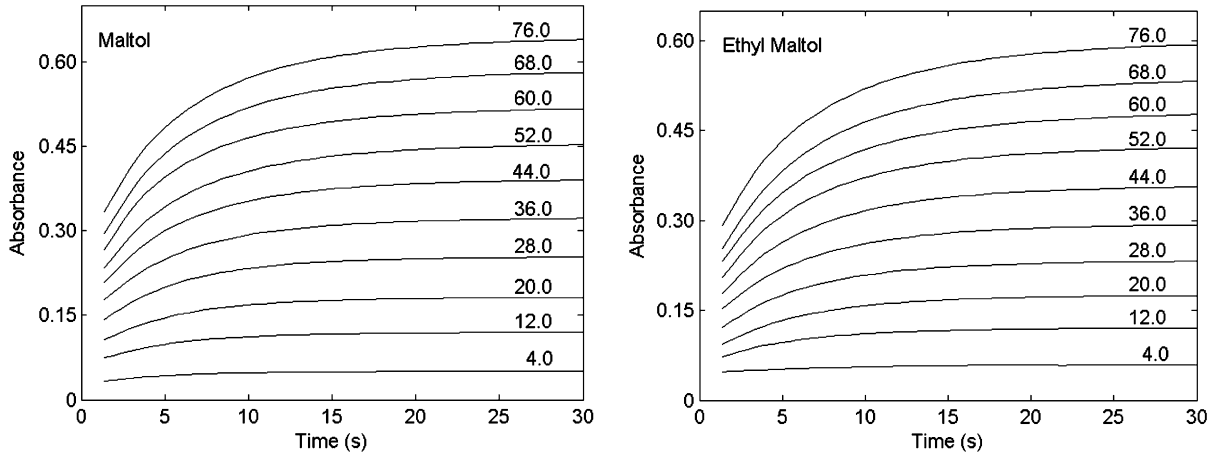

Fig. 3. Kinetic curves for maltol and ethyl maltol with different concentrations (mg L^{-1}). Experimental conditions are as in Fig. 1B.

Table 1
Comparison of verification results for synthetic samples by PCR, PLS and PC–RBF–ANN calibration models

Chemometrics methods	% RPE_S		% RPE_T
	Maltol	Ethyl maltol	
PCR (2)[a]	2.8 (103)[c]	6.6 (106)	5.1
PLS (2)[a]	2.8 (103)	6.6 (106)	5.1
PC–RBF–ANN (2, 3, 300)[b]	5.4 (106)	8.0 (109)	6.8

[a] Values in parentheses = number of factors used for PCR and PLS models.
[b] Values in parentheses = number of factors, nodes in the hidden layer and the spread coefficient (sc), respectively.
[c] Values in parentheses = mean recoveries (%).

($n = 10$, $r = 0.9998$) for ethyl maltol. The linear concentration range for both maltol and ethyl maltol was from 4.0 to 76.0 mg L^{-1}. The limits of detection (LODs) values, calculated according to the Miller's method (Miller, 1991), were 1.6 and 1.4 mg L^{-1} for maltol and ethyl maltol, respectively. Both the linear ranges and the LODs compare well with the figures of merit given by the HPLC methods, which utilize a UV detector (Liu et al., 2006).

4.4. Multivariate calibrations and their verification with synthetic mixtures

A calibration set of 11 samples and a verification set of 9 samples were prepared with the use of the three level orthogonal design denoted by $OA_9(3^2)$. The levels corresponded to values in the range of 4.0–64.0 mg L^{-1} for both maltol and ethyl maltol. For the PCR, PLS and PC–RBF–ANN calibration models, it was observed that there were no significant differences in the PRESS values beyond two factors according to the proposed method (Haaland & Thomas, 1988). These calibration models were verified with the verification set of 9 samples (Table 1). PCR and PLS models gave the same results with % RPE_S of 2.8 and % recovery of 103 for maltol, % RPE_S of 6.6 and % recovery of 106 for ethyl maltol, and % RPE_T of 5.1. The PC–RBF–ANN model produced similar but slightly worse figures of merit.

4.5. Selectivity

To study the selectivity of the proposed method, the effect of various substances on the analysis of a mixture of maltol (20.0 mg L^{-1}) and ethyl maltol (20.0 mg L^{-1}) was tested under the optimum conditions. The tolerance limit was defined as the concentration of added species causing less than ±10% relative error. The results showed that sucrose, glucose, critric acid, Na, K, and Ca ions did not interfere at a 500:1 interferent-to-anlyte concentration ratio; synthetic colorants, such as sunset yellow, tartrazine, amaranth, ponceau 4R, brilliant blue and indigo carmine produced interferences at about 200-fold concentration

Table 2
Maltol and ethyl maltol in food samples – PCR, PLS, PC–RBF–ANN calibrations, and HPLC-UV

Samples[a]	Found (μg g^{-1})[b] Original sample		Added (μg g^{-1}) Standard addition		Found (μg g^{-1})[b] Standard addition		% Recovery[c]	
	Maltol	Ethyl maltol	Maltol	Ethyl maltol	Maltol	Ethyl maltol	Maltol	Ethyl maltol
PCR								
1	20.1 ± 0.1	ND	5.0	10.0	24.9 ± 0.1	9.9 ± 0.2	96	99
2	14.7 ± 0.1	ND	10.0	5.0	24.6 ± 0.1	4.9 ± 0.1	99	98
3	ND[d]	19.7 ± 0.1	5.0	5.0	5.1 ± 0.1	24.4 ± 0.1	102	94
4	18.9 ± 0.1	ND	10.0	10.0	28.7 ± 0.2	10.0 ± 0.1	98	100
PLS								
1	20.1 ± 0.1	ND	5.0	10.0	24.9 ± 0.1	9.8 ± 0.2	96	98
2	14.7 ± 0.1	ND	10.0	5.0	24.6 ± 0.1	4.9 ± 0.1	99	98
3	ND	19.7 ± 0.1	5.0	5.0	5.1 ± 0.1	24.4 ± 0.1	102	94
4	18.9 ± 0.2	ND	10.0	10.0	28.7 ± 0.1	9.9 ± 0.1	98	99
PC–RBF–ANN								
1	19.7 ± 0.2	ND	5.0	10.0	24.8 ± 0.2	10.4 ± 0.1	102	104
2	13.6 ± 0.2	ND	10.0	5.0	25.1 ± 0.1	5.3 ± 0.1	115	106
3	ND	19.1 ± 0.1	5.0	5.0	5.1 ± 0.2	24.4 ± 0.2	102	106
4	18.4 ± 0.1	ND	10.0	10.0	29.9 ± 0.1	10.5 ± 0.2	115	105
HPLC								
1	20.0 ± 0.2	ND	5.0	10.0	24.7 ± 0.1	10.2 ± 0.2	94	102
2	13.8 ± 0.1	ND	10.0	5.0	23.9 ± 0.2	5.2 ± 0.1	101	104
3	ND	19.5 ± 0.2	5.0	5.0	5.2 ± 0.02	24.3 ± 0.1	104	96
4	19.2 ± 0.1	ND	10.0	10.0	29.5 ± 0.1	9.6 ± 0.2	103	96

[a] Samples: (1) Biscuits – Yichang Huaer Food Production Co. Ltd.; (2) Jelly – Anhui Jintianyuan Food Production Co. Ltd.; (3) Beverage – Shanxi Hengxing Fruitjuice Drink Production Co. Ltd., and (4) Julep – Hubei Yiding Julep Production Co. Ltd.
[b] Mean (replicates) ± standard deviation.
[c] % Recovery = $100 \times [(c_{Found(std.)} - c_{Found})/c_{Added}]$.
[d] ND = Not detected.

level relative to that of the flavour enhancers. Vanillin, ethyl vanillin, Zn(II), Mn(II), Pb(II), Cu(II) and Fe(III) produced only small effects even at a 100:1 interferent-to-analyte ratio, but ascorbic acid interfered seriously with the determination. Thus, it is important to extract the analytes from food samples with chloroform as suggested in Section 3.5. This minimizes such interferences.

4.6. Maltol and ethyl maltol determination in food samples

The verified calibrations for the PCR, PLS and PC–RBF–ANN methods were applied to the simultaneous determination of maltol and ethyl maltol in several food samples. There is a good agreement between the results (Table 2) from the multivariate calibrations, and those produced by the HPLC-UV method. The % recoveries are uniformly consistent. This indicates that multivariate prediction models can produce satisfactory results for the simultaneous determination of maltol and ethyl maltol in complex food samples.

5. Conclusion

A kinetic-spectrophotometric method has been researched and developed for the simultaneous determination of the common food flavour enhancing compounds, maltol, and its synthetic homologue, ethyl maltol. The significance of this method is that it enables a simple quantitative discrimination of the two compounds. This is important since biological health and safety concerns have been raised about ethyl maltol.

The suggested method utilizes the multivariate kinetic approach because the UV–visible spectra of the two compounds are almost the same. An indirect method, which follows the development of the pink colour of the ferroin reagent in the reaction with the two analytes, facilitates the discrimination of the compounds on a kinetic basis provided chemometrics modeling is applied for data interpretation.

Verified PCR, PLS and PC–RBF–ANN calibration models produced satisfactory results for the discrimination and prediction of the two analytes in synthetic mixtures, and in a range of food products. The results compare well with the analysis of the same samples by the HPLC-UV method.

Acknowledgements

The authors gratefully acknowledge the financial support of this study by the Natural Science Foundation of China (NSFC20365002 and 20562009), the State Key Laboratories of the Electroanalytical Chemistry of Changchun Applied Chemical Institute (SKLEAC2004-3) and the Chemo/Biosensing and Chemometrics of Hunan University (SKLCBC2005-22), the Jiangxi Province Natural Science Foundation (JXNSF0620041), and the program for Changjiang Scholars and Innovative Research Team in Universities (IRT0540).

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