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Partial Least Squares Method for Simultaneous Spectrophotometric Determination of Four Food Pigments

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Due to the strong overlapping of absorption spectra, the simultaneous determination of four food pigments mixtures (amaranth, carmine, enticed red, pyrosine) by using spectrophotometric method is difficult. A chemometrics method, partial least squares (PLS), was described for the simultaneous determination of four pigments in synthetic samples. Spectra of mixtures of four food pigments between 380-640 nm wavelengths by 0.2 nm intervals were recorded, in the range of concentrations comprised between 1-8 μg mL^{-1} for amaranth, carmine, enticed red and 0.5-4 μg mL^{-1} for pyrosine, respectively. The analyte recoveries from synthetic mixtures range from 100.26 to 103.24 % for amaranth, from 93.677 to 113.03 % for carmine, from 95.749 to 99.448 % for enticed red and from 85.446 to 104.33 % for pyrosine, respectively. The root mean square difference (RMSD) for the four analytes are 0.052772, 0.079997, 0.088947, 0.061912, respectively. The result shows that PLS is an efficient chemometrics method applied for prediction of the four analytes.

Key Words: Partial least squares, Spectrophotometric, Simultaneous determination, Food pigments.

INTRODUCTION

Colour is an important quality attribute of food products, being a determinant of its acceptability. In the food industry, food pigments are used in colourless food products as well as to compensate for colour lost as a result of processing conditions (*e.g.*, high temperature and pressure)[1]. They are used for maintenance and improvement of colour appearance in foods[2]. Food pigments allow capturing the desired esthetic quality of a particular food and this is the reason of the importance of food colouring in food industries. Because some synthetic pigments may be pathogenic, especially if they are consumed in excess[3], their determination is necessary.

The most frequently analytical technique, for the analysis of food pigments, is high-performance liquid chromatography[4-6]. However, this technique is expensive, labourious, time and use of large amount of organic solvents. The simultaneous determination of food pigments mixtures by using spectrophotometric method is a

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difficult problem in analytical chemistry, due to spectral interferences. By multivariate calibration methods, such as partial least squares (PLS) regression, it is possible to obtain a model adjusted to the concentration values of the mixtures used in the calibration range.

Partial least squares is a chemometrics technique which have been widely used for multivariate calibration in different analytical methods[7-10]. Its basic concept, detailed mathematical treatments and tutorials for chemical applications may be found in some literature[11-14]. The application of the quantitative chemometric method needs a calibration step where the relationship between the spectra and the component concentration is deduced from a set of reference samples, followed by prediction step in which the results of the calibration are used to determine the component concentration from the sample spectrum[15].

In this paper, a simple and sensitive method for the simultaneous determination of four food pigments mixtures (amaranth, carmine, enticed red, pyrosine) combining the advantages of spectrophotometric and multivariate calibration methods (PLS) with no previous separation steps is described.

EXPERIMENTAL

The spectrometric measurements were carried out by using Shimadzu UV-260 ultraviolet-visible spectrophotometer.

Partial least squares calculations were performed using programs developed in our own laboratory with the MATLAB, Version 7.0 high-level programming language and connected to a PC microcomputer.

Amaranth, carmine, enticed red and pyrosine stock solutions (500 μg mL^{-1}) were prepared weighing the required amount of the corresponding compounds (amaranth, carmine, enticed red and pyrosine) and dissolved in doubly distilled water.

Known amounts of the stock solutions were placed in a 25 mL volumetric flask and completed to the final volume with doubly distilled water. Spectrophotometric measurements were carried out with a Shimadzu UV-260 ultraviolet-visible spectrophotometer, employing a 10 mm quartz cell. Spectra of mixtures of four food pigments between 380-640 nm wavelengths by 0.2 nm intervals against a blank of solvent were recorded, in the range of concentrations comprised between 1-8 μg mL^{-1} for amaranth, carmine, enticed red and 0.5-4 μg mL^{-1} for pyrosine, respectively and then the data were stored for late treatment.

RESULTS AND DISCUSSION

Absorption spectra: The absorption spectra of amaranth,carmine,enticed red and pyrosine (concentration of each component is 8 μg mL^{-1}) are plotted in Fig. 1.

As we can see in the figure, the absorption spectra show absorption maxima located at 520 nm for amaranth, 506 nm for carmine, 504 nm for enticed red and 526 nm for pyrosine. There is also a clear overlapping of the four spectra. This

prevents the simultaneous determination of the four food pigments by spectrophotometric measurements without any separation process. By multivariate calibration methods, their quantitative determination without any separation process is feasible.

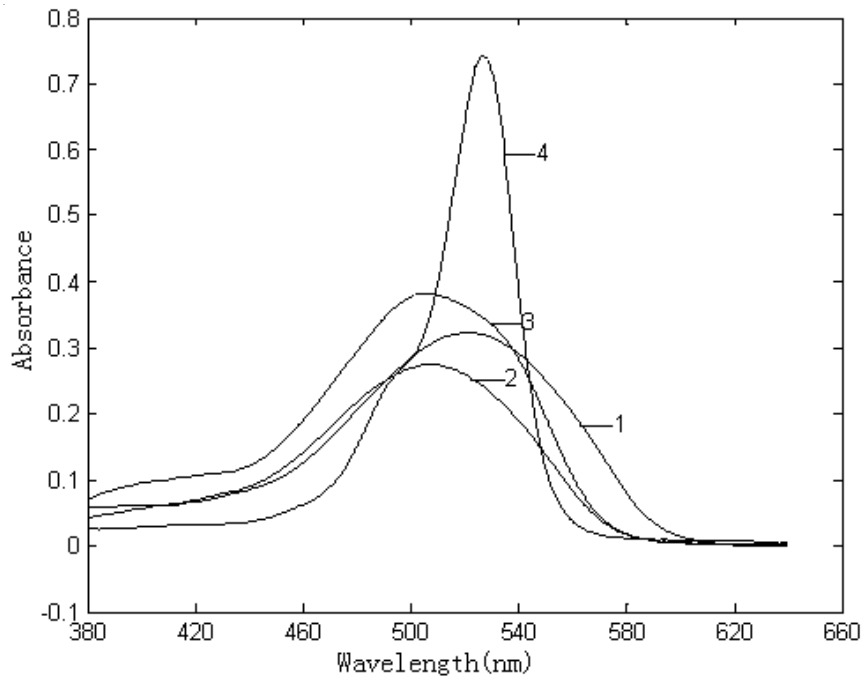

Fig. 1. Absorption spectra for: (1) amaranth; (2) carmine; (3) enticed red; (4) pyrosine, concentration of each component is 8 μg mL^{-1}

Preparation of the calibration and validation sets: A set of 32 samples was taken (Table-1). The concentrations comprised between 1-8 μg mL^{-1} for amaranth, carmine, enticed red and 0.5-4 μg mL^{-1} for pyrosine, respectively.

A quantity of mixtures from M25 to M32 was selected as prediction set. All or some of the mixtures from M1 to M24 were selected as calibration set.

Selection of optimum number of factors: The optimum number of factors was determined by computing the prediction residual error sum of square (PRESS) for cross-validated models using a high number of factors[16]. The PRESS values provide a measure of how well the training set is predicting the concentration for each number of factors. Fig. 2 shows the PRESS against the number of factors for each individual component, selecting M1-M24 as calibration set, spectra of mixtures of four food pigments between 380-640 nm wavelengths by 4 nm intervals. For finding the smallest model (fewest number of factors), the F-statistic was used to carry out the significance determination. In all instances, the number of factors for the first PRESS values whose F-ratio probability drops below 0.75 was selected as the optimum[17].

TABLE-1
CONCENTRATION DATA OF THE DIFFERENT MIXTURES OF FOUR FOOD PIGMENTS

Mixture	Amaranth ($\mu g\ mL^{-1}$)	Carmine ($\mu g\ mL^{-1}$)	Enticed red ($\mu g\ mL^{-1}$)	Pyrosine ($\mu g\ mL^{-1}$)
M1	1.0	8.0	1.0	4.0
M2	2.0	8.0	3.0	4.0
M3	3.0	8.0	5.0	3.0
M4	4.0	8.0	7.0	3.0
M5	5.0	7.0	2.0	2.0
M6	6.0	7.0	4.0	2.0
M7	7.0	7.0	6.0	1.0
M8	8.0	7.0	8.0	1.0
M9	1.0	6.0	1.0	3.5
M10	2.0	6.0	3.0	3.5
M11	3.0	6.0	5.0	2.5
M12	4.0	6.0	7.0	2.5
M13	5.0	5.0	2.0	1.5
M14	6.0	5.0	4.0	1.5
M15	7.0	5.0	6.0	0.5
M16	8.0	5.0	8.0	0.5
M17	1.0	4.0	1.0	4.0
M18	2.0	4.0	3.0	4.0
M19	3.0	4.0	5.0	3.0
M20	4.0	4.0	7.0	3.0
M21	5.0	3.0	2.0	2.0
M22	6.0	3.0	4.0	2.0
M23	7.0	3.0	6.0	1.0
M24	8.0	3.0	8.0	1.0
M25	1.0	2.0	1.0	3.5
M26	2.0	2.0	3.0	3.5
M27	3.0	2.0	5.0	2.5
M28	4.0	2.0	7.0	2.5
M29	5.0	1.0	2.0	1.5
M30	6.0	1.0	4.0	1.5
M31	7.0	1.0	6.0	0.5
M32	8.0	1.0	8.0	0.5

According to the result shows in Fig. 2, the optimal number of factors for amaranth, carmine, enticed red and pyrosine was 5, 6, 5 and 2, respectively.

Selection of calibration set: A quantity of mixtures from M25 to M32 was selected as prediction set. The mixtures from M1 to M8, M1 to M12, M1 to M16, M1 to M20 and M1 to M24 were selected as calibration sets, respectively. The root mean square difference (RMSD) was calculated for each component. The values of RMSD are showed in Table-2.

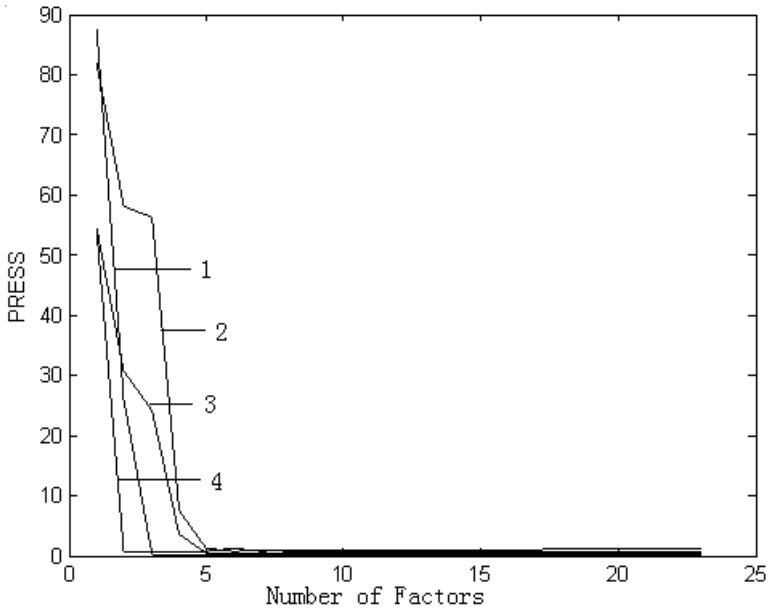

Fig. 2. PRESS against the number of factors for (1) amaranth; (2) carmine; (3) enticed red; (4) pyrosine

TABLE-2
ROOT MEAN SQUARE DIFFERENCE (RMSD) IN DIFFERENT CALIBRATION SETS*

Calibration set	Root mean square difference			
	Amaranth	Carmine	Enticed red	Pyrosine
M1 to M8	0.042842	1.24340	0.952180	0.052142
M1 to M12	0.091770	0.22475	0.133800	0.864000
M1 to M16	0.029531	0.21984	0.076999	0.551540
M1 to M20	0.050471	0.23111	0.218580	0.066094
M1 to M24	0.052273	0.15422	0.146110	0.056608

*Selecting the number of factors for all the four food pigments as 5, spectra of mixtures of four food pigments between 380-640 nm wavelengths by 0.2 nm intervals.

Table-2 shows the selection of the calibration set is very important for achieving the best prediction. With the increase in mixtures of the calibration sets, RMSD gets smaller. This may be because more mixtures can provide more useful information. In order to get the least RMSD, M1 to M24 was selected as calibration set.

Selection of the wavelength range: In multi-component spectrophotometric analysis, the optimum wavelength range should be carefully chosen for getting acceptable accuracy and precision in the results.

To select the optimum spectral region for the analysis, the root mean square difference (RMSD) was calculated for each component in the range of wavelength 500-520, 460-560, 420-600 and 380-640 nm, respectively. The values of RMSD are showed in Table-3.

TABLE-3
ROOT MEAN SQUARE DIFFERENCE IN DIFFERENT WAVELENGTH RANGE*

Wavelength range (nm)	Root mean square difference			
	Amaranth	Carmine	Enticed red	Pyrosine
500-520	1.122600	2.56140	1.10240	0.120330
460-560	0.066483	0.34514	0.22018	0.057240
420-600	0.058935	0.20753	0.11259	0.068996
380-640	0.052273	0.15422	0.14611	0.056608

*Selecting the number of factors for all the four food pigments as 5, spectra of mixtures of four food pigments by 0.2 nm intervals.

Table-3 shows that as the range of wavelength increases, RMSD gets smaller. This may be because a wider wavelength range can provide more useful information. In the range of wavelength 380-640 nm, there are relatively low RMSD. So this region was adapted.

Selection of the wavelength interval: To select the optimum wavelength interval for the analysis, the root mean square difference (RMSD) by different intervals was calculated. The values of RMSD are showed in Table-4.

TABLE-4
ROOT MEAN SQUARE DIFFERENCE AT DIFFERENT WAVELENGTH INTERVALS*

Wavelength intervals (nm)	Root mean square difference			
	Amaranth	Carmine	Enticed red	Pyrosine
0.2	0.052273	0.15422	0.14611	0.056608
1.0	0.050489	0.15247	0.14161	0.05978
2.0	0.054583	0.13088	0.13018	0.059895
4.0	0.052772	0.079997	0.088947	0.061912
6.0	0.048477	0.15644	0.146	0.060466
8.0	0.055384	0.13277	0.11495	0.066309

*Selecting the number of factors for all the four food pigments as 5, for each component in the range of wavelength 380-640 nm.

Table-4 shows the selection of the wavelength intervals is very important for achieving the best prediction. Too large or too small wavelength intervals have caused an increase in RMSD. This may be due to too large intervals may lose some useful information, while too small intervals may result in more noise. In order to get the least RMSD, 4nm was selected as wavelength intervals.

Application to synthetic mixtures: The root mean square difference (RMSD) and recovery was calculated for each component. The values of RMSD and recovery are showed in Table-5.

Table-5 shows that the analyte recoveries from synthetic mixtures range from 100.26 to 103.24 % for amaranth, from 93.677 to 113.03 % for carmine, from 95.749 to 99.448 % for enticed red and from 85.446 to 104.33 % for pyrosine, respectively, while the RMSD for the four food pigments are 0.052772, 0.079997, 0.088947, 0.061912, respectively.

TABLE-5
RECOVERY AND ROOT MEAN SQUARE DIFFERENCE OF THE SYNTHETIC MIXTURES*

Solution number	Recovery			
	Amaranth	Carmine	Enticed red	Pyrosine
M25	103.24	100.84	95.833	99.363
M26	101.12	102.04	98.729	99.268
M27	102.35	95.675	99.440	97.527
M28	101.47	102.45	98.486	95.000
M29	101.23	95.136	98.049	103.21
M30	101.18	112.66	95.749	102.97
M31	100.80	93.677	99.448	104.33
M32	100.26	113.03	98.392	85.446
RMSD	0.052772	0.079997	0.088947	0.061912

*Selecting the number of factors for all the four food pigments as 5, M1 to M24 as calibration set, spectra of mixtures of four food pigments between 380-640 nm wavelengths by 4 nm intervals. RMSD = Root mean square difference.

Conclusion

According to these studies, it is concluded that partial least squares method is suitable for resolving overlapping absorption spectra of mixtures of amaranth, carmine, enticed red and pyrosine with satisfactory results. Compared with traditional spectrophotometric and high-performance liquid chromatography, this method is convenient, fast and efficient.

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