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Resolution of kinetic system of simultaneous degradations of chlorophyll *a* and *b* by PARAFAC

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Abstract

A three-way resolution method based on PARAFAC analysis of fluorescence excitation/emission matrices (EEMs) is presented to study the black kinetic system of simultaneous degradations of chlorophyll *a* and *b* extracted with other interferents from fresh spinach. The excitation and emission spectral profiles as well as the kinetic concentration profiles of chlorophyll *a*, *b* and their degradation products, pheophytin a, b were resolved for this multi-component kinetic system. The degradation of chlorophyll to pheophytin under the optimized experimental conditions is confirmed to follow the first-order reaction model. The most obvious advantage of this method is that the complex black multi-component kinetic system can be resolved and studied in the presence of unknown interferents by applying PARAFAC, which is capable of resolving the three-way data array provided by EEMs and giving a unique solution to the analytical problem. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Black system; Chlorophyll degradation; Kinetics; PARAFAC; Pheophytin; Three-way resolution; Trilinear model

1. Introduction

There is widespread interest in chlorophyll and their degradation reactions in many fields of science. For example: the research of plant pigments by Tswett, a Russian botanist, resulted in the advent of chromatography [1]; the origin of organic geochemistry was attributed to the Treibs hypothesis that petroporphyrins originate from chlorophyll [2,3]; biochemist and biophysicist show great interest in the mechanism of photosynthesis that is related to chlorophyll [4]; also, the chlorophyll degradation products are often used by environmental scientist to monitor the fate of algae [3,5], and so on.

In agriculture and industry, the study of color change from bright green to olive brown during storage and processing is of major interest and importance for technologists and researchers due to the fact that color, the major quality attribute of vegetables and fruits, may finally make the consumer decide whether buy them or not [6,7]. Therefore, many researchers make great effort to elucidate the mechanism of degradation from chlorophyll to pheophytin formed by substitution of hydrogen ion to magnesium ion in the porphyrin ring of chlorophyll [8], which is well known to be the main cause of loss of green color in coleslaw [8], green leaves [9], asparagus [10], and olive [11] (the structures of chlorophyll *a* and *b* are shown in Fig. 1).

By now, to our knowledge, most of the traditional studies on degradation reaction of chlorophyll have

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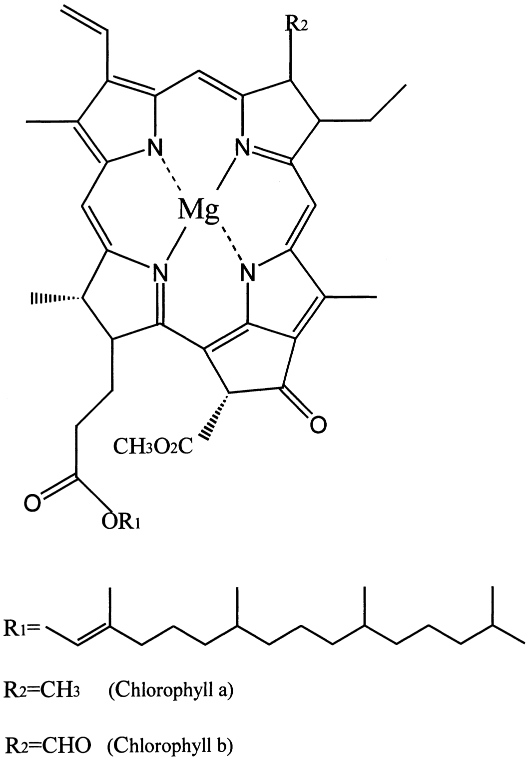

Fig. 1. The structure of chlorophyll *a*, *b*.

been conducted either with chromatography to separate the pigments followed by spectrophotometric determination of each pigment of interest [7,11], or by measuring the spectrophotometric response of treated mixture of pigments at different specific wavelengths, then solving simultaneous equations to determine the concentrations [8,10]. In chromatography, the complete separation is difficult to achieve, for the eluting peaks are very close to each other [3] and at the same time the kinetic system is perturbed. The sensitivity of spectrophotometric methods is, unfortunately, rather low and the measurement is interfered by co-existing pigments and other interferents [12]. Also, they are time-consuming and tedious.

In the last two decades analyst can obtain multi-dimentional data array provided by so-called ‘hyphenated’ instruments such as a high-performance liquid chromatograph with a diode array detector (HPLC-DAD), or an excitation/emission matrix (EEM) spectrofluorometer. This usually means that analysis can be performed in the presence of unknown interferents, which is called the ‘second-order advantage’ [13,14]. Thus far, several algorithms have been developed capable of exploiting the ‘second-order advantage’ such as the generalized rank annihilation method (GRAM) which was proposed by E. Sanchez and B.R. Kowalski [15,16], or parallel factor analysis (PARAFAC) developed and popularized by Harshman [17] which utilizes alternating least squares in an iterative procedure that exploits the conditional linearity of the trilinear model. These algorithms have been used to solve some complex chemical problems [18,19].

Most of the applications of these chemometric algorithms are focused on calibration which entails the standards of analytes of interest [18,20,21]. In analytical practice, unfortunately, there are many important cases where the calibration can not be performed because there is no previous information available about the system [22], which is a so-called black one [23]. The application of three-way resolution methods to the study of black kinetic system has been rarely reported in spite of the potential of these methods for the elucidation of mechanism of kinetic process [24]. This paper reports a study of the black multi-component kinetic system of chlorophyll degradation by applying PARAFAC, which can give the unique solution of the problem by resolving the three-way data array provided by EEMs.

In this study, the mixture of pigments including chlorophyll *a*, *b* and other interferents was extracted firstly from fresh spinach by an organic solvent. Next, the simultaneous degradation reactions of chlorophyll *a* and *b* were initiated by adding the extract to an acidic buffer. Thereafter, the reaction was interrupted by rapidly declining the concentration of hydrogen ion at different specific moments and corresponding excitation/emission matrices (EEMs) were measured sequentially. Finally, the PARAFAC algorithm was applied to resolve the excitation and emission spectral profiles as well as the kinetic concentration profiles of chlorophyll *a*, *b* and pheophytin a, b for three-way data array provided by EEMs.

The objective of this research is to: (1) provide a new convenient and time-saving method for the kinetic studies on simultaneous degradations of chlorophyll *a* and *b* in the presence of interferents; (2) give a real-world example of resolution of a complex multicomponent kinetic system in the presence of interferents by applying PARAFAC to analyze the three-way data array provided by EEMs.

2. Theory

2.1. Trilinear model

When the kinetic reactions of a mixture are monitored using spectrofluorometry on I excitation wavelengths and J emission wavelengths at K moments, a set of EEMs are obtained and collected in an $I \times J \times K$ three-way data array $\underline{\boldsymbol{X}}$. A trilinear model for such a three-way data array $\underline{\boldsymbol{X}}$ has the form:

$$\underline{\boldsymbol{X}} = \sum_{n=1}^{N} \boldsymbol{a}_n \otimes \boldsymbol{b}_n \otimes \boldsymbol{c}_n + \underline{\boldsymbol{E}} \quad (1)$$

where N denotes the number of detectable species, comprised of components of interest and the background components including interferents, $\otimes$ denotes the tensor product, and $\boldsymbol{a}_n$ is the excitation spectral profile of the nth component; $\boldsymbol{b}_n$ is the emission spectral profile of the nth component; $\boldsymbol{c}_n$ is the kinetic concentration profile of the nth component. $\underline{\boldsymbol{E}}$ is the three-way array of measurement residuals.

In matrix notation the trilinear model can be written as

$$\boldsymbol{X}_{..k} = \boldsymbol{A}\,\mathrm{diag}(\boldsymbol{c}_{(k)})\boldsymbol{B}^{\mathrm{T}} + \boldsymbol{E}_{..k} \quad k = 1, 2, \ldots, K \quad (2)$$

where $\boldsymbol{X}_{..k}$ and $\boldsymbol{E}_{..k}$ are the kth slices of $\underline{\boldsymbol{X}}$ and $\underline{\boldsymbol{E}}$, respectively, along the kinetic concentration order; $\boldsymbol{A} = (\boldsymbol{a}_1, \boldsymbol{a}_2, \ldots, \boldsymbol{a}_N)$, $\boldsymbol{B} = (\boldsymbol{b}_1, \boldsymbol{b}_2, \ldots, \boldsymbol{b}_N)$ and $\boldsymbol{c}_{(k)}$ denotes the kth row of $\boldsymbol{C} = (\boldsymbol{c}_1, \boldsymbol{c}_2, \ldots, \boldsymbol{c}_N)$, and diag $(\boldsymbol{c}_{(k)})$ denotes the diagonal matrix of order $N \times N$ in which the corresponding diagonal elements are elements of $\boldsymbol{c}_{(k)}$. The superscript T denotes the transpose of the matrix.

2.2. PARAFAC algorithm

In the standard PARAFAC algorithm, the loss function to be minimized is the sum of squared residuals (SSR), which may be written as

$$\mathrm{SSR} = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} e_{ijk}^2 \quad (3)$$

Where e_{ijk} denotes the i, j, kth residual element of $\underline{\boldsymbol{E}}$.

The solution for PARAFAC model can be found by alternating least squares algorithm (ALS). A typical iterative procedure is given as follows:

1. Decide on the number of components N.
2. Initialize $\boldsymbol{A}$ and $\boldsymbol{B}$.
3. Estimate $\boldsymbol{C}$ from $\underline{\boldsymbol{X}}$, $\boldsymbol{A}$ and $\boldsymbol{B}$ as:

$$\boldsymbol{c}_{(k)}^{\mathrm{T}} = (\boldsymbol{A}^{\mathrm{T}}\boldsymbol{A} * \boldsymbol{B}^{\mathrm{T}}\boldsymbol{B})^{-1}\mathrm{diag}(\boldsymbol{A}^{\mathrm{T}}\boldsymbol{X}_{..k}\boldsymbol{B})1, \quad k = 1, \ldots, K \quad (4)$$

Where **1** denotes the N-dimensional vector with each element being 1 and * denotes the Hadamard product, that is, if $\boldsymbol{C}_{M\times N} = \boldsymbol{A}_{M\times N} * \boldsymbol{B}_{M\times N}$, then $c_{mn} = a_{mn} \times b_{mn}$.

4. Estimate $\boldsymbol{A}$ from $\underline{\boldsymbol{X}}$, $\boldsymbol{B}$ and $\mathbf{C}$ as:

$$\boldsymbol{A} = \left(\sum_{k=1}^{K}\boldsymbol{X}_{..k}\boldsymbol{B}\,\mathrm{diag}(\boldsymbol{c}_{(k)})\right) \times \left(\sum_{k=1}^{K}\mathrm{diag}(\boldsymbol{c}_{(k)})\boldsymbol{B}^{\mathrm{T}}\boldsymbol{B}\,\mathrm{diag}(\boldsymbol{c}_{(k)})\right)^{-1} \quad (5)$$

5. Estimate $\boldsymbol{B}$ from $\underline{\boldsymbol{X}}$, $\boldsymbol{A}$ and $\boldsymbol{C}$ as:

$$\boldsymbol{B} = \left(\sum_{k=1}^{K}\boldsymbol{X}_{..k}^{\mathrm{T}}\boldsymbol{A}\,\mathrm{diag}(\boldsymbol{c}_{(k)})\right) \times \left(\sum_{k=1}^{K}\mathrm{diag}(\boldsymbol{c}_{(k)})\boldsymbol{A}^{\mathrm{T}}\boldsymbol{A}\,\mathrm{diag}(\boldsymbol{c}_{(k)})\right)^{-1} \quad (6)$$

6. Iterate from (2) to (5) until convergence.

In this presented study, $\boldsymbol{A}$, $\boldsymbol{B}$ is initialized by the first N singular vectors of $\sum_{k=1}^{K}\boldsymbol{X}_{..k}\boldsymbol{X}_{..k}^{\mathrm{T}}$ and $\sum_{k=1}^{K}\boldsymbol{X}_{..k}^{\mathrm{T}}\boldsymbol{X}_{..k}$, respectively. The stopping criterion is that the improvement of the SSR between the consecutive iterations is less than 10^{-8}.

3. Material and methods

3.1. Apparatus

HITACHI 850 florescence spectrometer (Hitachi, Japan); PHS-3C digital pH-meter (REX, Shanghai, China); glass electrode and Ag/AgCl (saturated KCl) reference electrode.

3.2. Extraction of chlorophyll

Fresh spinach was obtained from a local supermarket. A 20 g sample of leaves without rib was shredded and blended with appropriate amount of acetone (analytical grade), then ground for 30 min in mortar by pestle, thereafter filtrated through filter paper. The first 20 ml of filtrate was collected in a 25 ml tube with a ground-glass plug. Finally, the extract was stored at −20°C until used although it was not kept for more than 24 h [12].

3.3. Preparation of buffer

All reagents were of analytical grade

1. Buffer A: This buffer was used to stabilize the concentration of hydrogen ion throughout the reaction. It was prepared by mixing 2 ml of HCI ($\mathrm{pH}=1.85$, which was the pH value of the HCl solution before mixed with acetone) with 18 ml of acetone.
2. Buffer B: This buffer was used to interrupt the reaction at a series of specific moments by rapidly declining the concentration of hydrogen ion to the extent that the concentration of chlorophyll *a*, *b* is far more than that of hydrogen ion. Buffer B was prepared by blending 50 ml of NaOH ($\mathrm{pH}=12.00$, which was the pH value of the NaOH solution before mixed with acetone) with 450 ml of acetone and 60 ml of double-distilled H_2O, sufficiently stirring by an agitator to obtain a homogenous acetone solution.

3.4. Experimental procedure

1. Initiation: Degradation reaction was initiated by adding 1 ml of chlorophyll extract to 20 ml of the buffer A whose temperature was preserved at 6°C throughout the testing.
2. Interruption: 1 ml of reaction mixture was taken out of reaction vessel to sufficiently mix with 24 ml of the buffer B whose temperature was also kept at 6°C after appropriate time intervals from 0 to 570 min (0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 10.5, 20.5, 30.5, 40.5, 50.5, 70.5, 120.5, 240.5, 360.5, 570.5 min).
3. Measurement: All 16 samples obtained by step (2) at different moments were measured with the florescence spectrometer (the Hitachi instrument parameters chosen for measurement were: Ex bandpass: 5 nm; Em bandpass: 5 nm; Scan speed: 120 nm/min; Response time: 2 s; PM Gain: high; EM filter: open). The excitation and the emission wavelengths were set from 300 to 500 and from 600 to 700 nm, respectively, with a fixed interval of 5 nm. The effect of Raleigh scattering on each response matrix was eliminated by subtracting the measurement matrix of a blank from that of samples [25]. A $41 \times 21 \times 16$ three-way data array was thereby collected.
4. Treatment of data: In order to eliminate the effect of the second scatter light, the first nine rows data (excitation wavelengths from 300 to 340 nm) were removed from each 41×21 matrix, producing a $32 \times 21 \times 16$ three-way data array. This data array was then treated using PARAFAC algorithm programmed with MATLAB on a personal computer (Pentium II 350 MHz) to resolve the profiles of components of interest.

4. Results and discussion

4.1. Optimization of experimental conditions

The temperature of reaction and measurement was chosen at 6°C for both reducing the rate of reaction and eliminating the effect of chlorophyllase, a kind of enzyme which can covert the chlorophyll to chlorophyllide and pheophytin to pheophorbide, respectively.

The pH value of the reaction mixture was set to 1.85 to make the degradation reaction of chlorophyll *b* almost complete within 570 min as well as make it possible to sample timely the degradation reaction of chlorophyll *a*, for the degradation rate of chlorophyll *a* was very high under the experimental conditions chosen in this study (degradation reaction of chlorophyll *a* completed within 30 min). The experiments could be also undertaken at higher pH values, though the reaction time might last many days.

Under the experimental conditions chosen in this study, since the high rate of the degradation reaction of chlorophyll *a* as well as the fact that the declining rate of the concentration in degradation reaction becomes more and more slow with the reaction proceeding, we sampled densely in the first 5.5 min (0.5, 1.5, 2.5, 3.5, 4.5, 5.5 min) to collect enough kinetic information about chlorophyll *a* and sparsely in the range from 10.5 to 30.5 min (10.5, 20.5, 30.5 min) to lessen our workload. Similarly, since the degradation reaction of chlorophyll *b* almost completed in 570 min, in addition to the nine sample points we picked up for chlorophyll *a*, we sampled densely in the range from 40.5 to 70.5 min (40.5, 50.5, 70.5 min) and sparsely in the range from 120.5 to 570.5 min (120.5, 240.5, 360.5, 570.5 min) to collect the kinetic information about chlorophyll *b*.

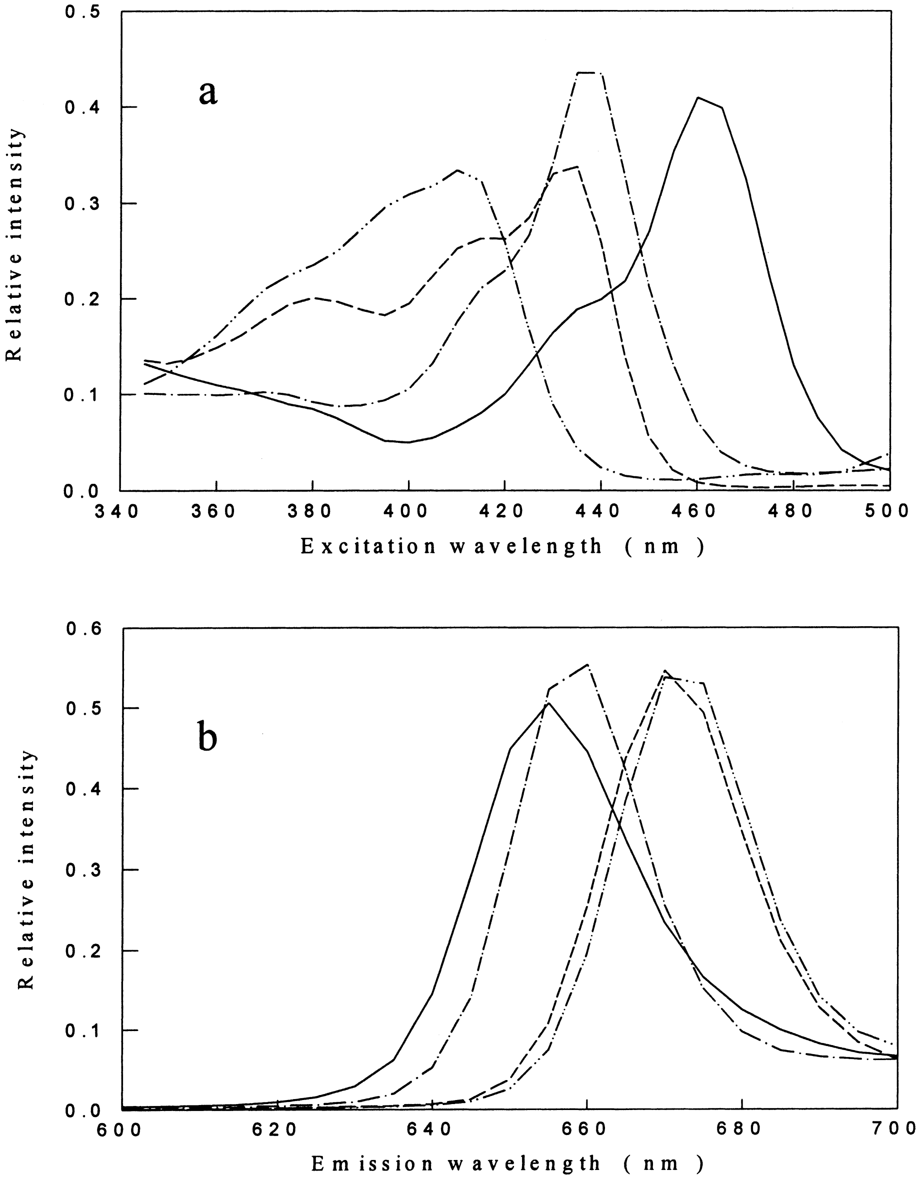

Fig. 2. Excitation and emission spectral profiles of chlorophyll *a*, *b* and pheophytin *a*, *b*. (a) Excitation spectral profiles of chlorophyll *a* (dash line), pheophytin a (dash double-doted line), chlorophyll *b* (full line) and pheophytin *b* (dash doted line). (b) Emission spectral profiles of chlorophyll *a* (dash line), pheophytin *a* (dash double-doted line), chlorophyll *b* (full line) and pheophytin *b* (dash doted line).

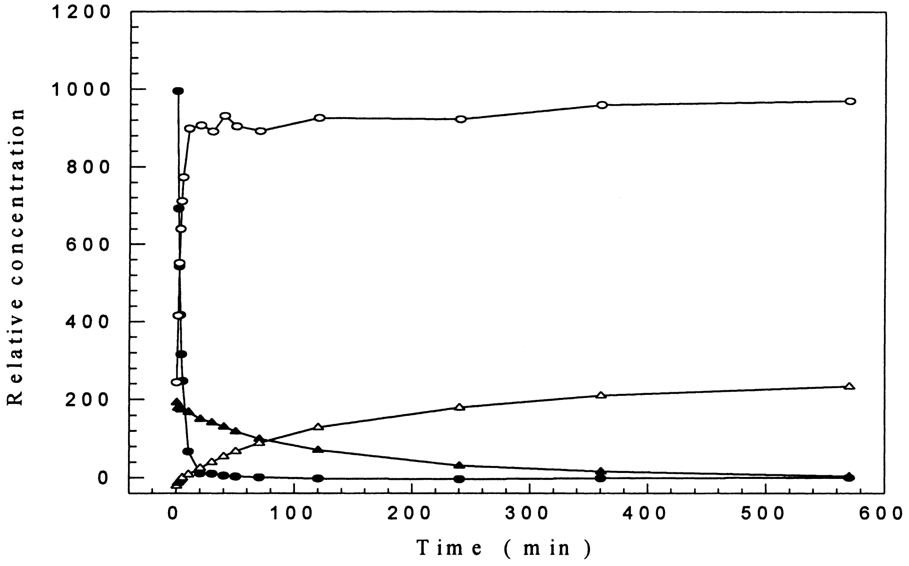

Fig. 3. The plot of kinetic concentration profiles of chlorophyll *a*, *b* and pheophytin *a*, *b* vs. reaction time. chlorophyll *a* (●), *b* (solid▲) and pheophytin *a* (○), *b* (△).

4.2. Resolution of black kinetic system

In analyzing the 32 × 21 × 16 three-way data array by PARAFAC, it was observed that there was little difference when the component number in the PARAFAC model was set to different number from 4 to 7. In contrast, if the 8-component PARAFAC model was considered, the degenerate solution was produced, indicating too many components were extracted [25]. Therefore, in this study, the component number in the PARAFAC model was chosen as 7.

The resolved excitation and emission spectral profiles as well as the kinetic concentration profiles of four main components of interest were shown in Figs. 2 and 3, respectively. By comparing the resolved spectral profiles for this kinetic system and the reported spectra from literature [12], it could be sure that these four components were chlorophyll *a*, *b* and their degradation products, pheophytin a and b, respectively.

From Fig. 2b, it could be observed that the emission spectral profile of chlorophyll *a* was almost completely overlapped with that of pheophytin a, indicating a strong collinearity among the emission spectral profiles. This meant that it was very difficult to resolve all the components of interest actually unless the obtained data had very high ratio of signal to noise. In this study, the good agreement between the resolved spectra profiles and the reported spectra profiles as well as the actual resolution of these two almost completely overlapped emission spectral profiles further confirmed the fact that a complex system which is very difficult to be analyzed using general chemical and simple mathematical methods can be resolved by a proper chemometric algorithm.

4.3. Validation of first-order kinetic model

If the conversion of chlorophyll to pheophytin followed first-order reaction kinetic model, it would be true that

$$\mathrm{Ln}\left(\frac{C_t}{C_0}\right) = -K_T \times t \qquad (7)$$

Where Ln(*) denoted the natural logarithm of *, C_0 was the initial chlorophyll concentration, K_T represented the reaction rate constant (min^{-1}) at a given temperature T°C, and C_t indicated the chlorophyll concentration at the moment t since initiation of reaction.

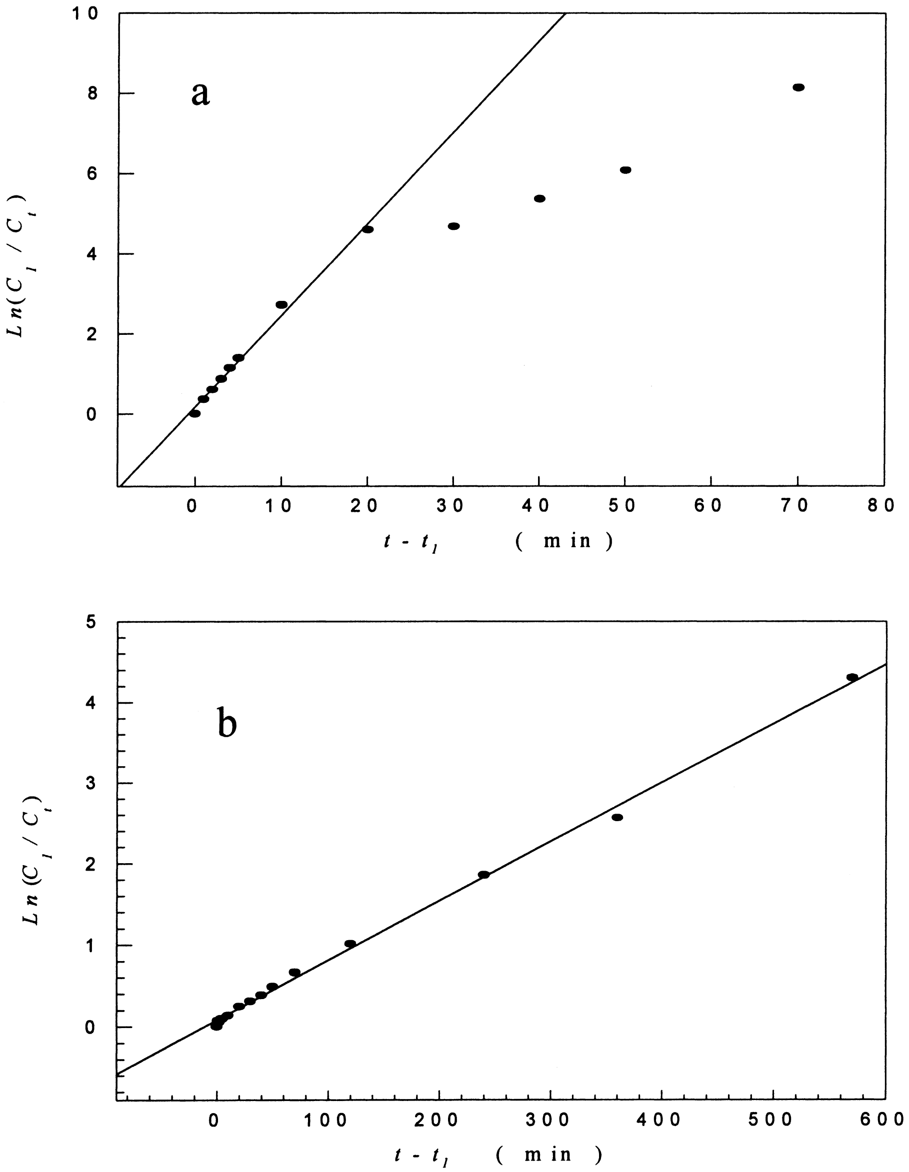

Fig. 4. The plot of the natural algorithm of relative concentration C_1/C_t vs. time $t-t_1$. (a) Chlorophyll *a*. (b) Chlorophyll *b*.

Table 1
Regression equation, rate constant and correlation coefficient of degradation reactions of chlorophyll *a* and *b*

Pigment	Regression equation	Rate constant (min^{-1})	Correlation coefficient
Chlorophyll *a*	$y^{\mathrm{a}} = 0.2286x^{\mathrm{a}} + 0.1755$	0.2286	0.9959
Chlorophyll *b*	$y = 0.0073x + 0.0749$	0.0073	0.9989

[a] y represents the natural logarithm of relative concentration of C_1 to C_t. x represents time $t-t_1$ (min).

Then one could get:

$$\mathrm{Ln}\left(\frac{C_1}{C_t}\right) = K_T \times (t - t_1) \quad (8)$$

In this case, C_1 represented the concentration of chlorophyll when the reaction was firstly interrupted at the moment $t_1 = 0.5$ min.

The logarithmic concentrations of the first 12 points of chlorophyll *a* versus time $t - t_1$ was plotted in Fig. 4a (the last 4 points were discarded due to the negative estimated concentrations). From this plot, it could be observed that the logarithmic concentrations of the first 8 point in the range from 0.5 to 20.5 min linearly related to time $t - t_1$ and the last 4 points in the range from 30.5 to 70.5 deviated from the linearity because the concentrations of these 4 points were near to zero such that the absolute errors of their logarithmic values were too great. Therefore, the first 8 points were used to regress and the regression equation and correlation coefficient of chlorophyll *a* was presented in Table 1.

The logarithmic concentration of 16 points of chlorophyll *b* versus the time $t - t_1$ was shown in Fig. 4b. It could be observed that all 16 points were almost in one line and that the first 6 points almost completely overlapped because the change of the concentration of chlorophyll *b* was little in the range from 0.5 to 5.5 min. The regression equation and correlation coefficient of chlorophyll *b* were shown in Table 1.

The high correlation coefficient shown in Table 1 (0.9959 for chlorophyll *a* and 0.9989 for chlorophyll *b*) confirmed that the degradation reaction of chlorophyll fit the first-order reaction kinetic model well. The fact that the reaction rate constant K_a for chlorophyll *a* shown in Table 1 was much larger than K_b for chlorophyll *b* indicated that the degradation of chlorophyll a was much faster than that of chlorophyll *b* which was also found previously by several investigators [7,11].

5. Conclusions

In this study, the kinetics of simultaneous degradations of chlorophyll *a* and *b* in extract from fresh spinach was studied by using PARAFAC analysis of three-way data array provided by EEMs. The experimental results revealed that the degradation reaction of chlorophyll fit first-order kinetic model. The presented paper provided not only a new efficient approach for food chemists to study the kinetics of chlorophyll in the practical conditions but also a promising tool for exploring kinetic behaviors of complex multi-components system which was of interest in physicochemical research.

Acknowledgements

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References

[1] A. Braithwaite, F.J. Smith, Chromatographic Methods, 4th Edition, Chapman & Hall, New York, 1985.
[2] A. Treibs, Angew. Chem. 49 (1936) 682.
[3] Y.-Z. Liang, R.G. Brereton, O.M. Kvalheim, A. Rahmani, Analyst 118 (1993) 779.
[4] J.W. Kimball, Biology, 4th Edition, Addison-Wesley, Reading, MA, 1979.
[5] J. Newton Downs, C.J. Lorenzen, Limnol. Oceanogr. 30 (1985) 1024.
[6] J.A. Steet, C.-H. Tong, J. Agric. Food Chem. 44 (1996) 1531.
[7] S.J. Schwartz, J.H. Von Elbe, J. Food Sci. 48 (1983) 1303.
[8] J.W. Heaton, R.Y. Yada, A.G. Marangoni, J. Agric. Food Chem. 44 (1996) 395.
[9] D.R. Haisman, M.W. Clarke, J. Sci. Food Agric. 26 (1975) 1111.
[10] S.S. Wang, N.F. Haard, G.R. Dimarco, J. Food Sci. 36 (1971) 657.
[11] M.I. Minguez-Mosquera, J. Garrido-Fernandez, B. Gandul-Rojas, J. Agric. Food Chem. 37 (1989) 8.
[12] K.G. Boto, J.S. Bunt, Anal. Chem. 50 (1978) 392.
[13] K.S. Booksh, B.R. Kowalski, Anal. Chem. 66 (1994) 782A.
[14] H.-L. Wu, M. Shibukawa, K. Oguma, J. Chemom. 12 (1998) 1.
[15] E. Sanchez, B.R. Kowalski, Anal. Chem. 58 (1986) 496.
[16] B. Wilson, E. Sanchez, B.R. Kowalski, J. Chemom. 3 (1989) 493.
[17] R.A. Harshman, UCLA Working Papers in Phonet, Vol. 16, 1970, p. 1.
[18] Y.-L. Xie, J.J. Baeza-Baeza, G. Ramis-Ramos, Chemom. Intell. Lab. Syst. 32 (1996) 215.
[19] P. Geladi, Chemom. Intell. Lab. Syst. 7 (1989) 11.
[20] J.L. Beltran, J. Guiteras, R. Ferrer, Anal. Chem. 70 (1998) 1949.
[21] A.K. Smilde, R. Tauler, J.M. Henshaw, L.W. Burgess, B.R. Kowalski, Anal. Chem. 66 (1994) 3345.
[22] R. Tauler, B.R. Kowalski, Anal. Chem. 65 (1993) 2040.
[23] Y.-Z. Liang, O.M. Kvalheim, R. Manne, Chemom. Intell. Lab. Syst. 18 (1993) 235.
[24] J. Saurina, S. Hernandez-Cassou, R. Tauler, A. Izquierdo-Ridorsa, J. Chemom. 12 (1998) 183.
[25] R. Bro, Chemom. Intell. Lab. Syst. 38 (1997) 149.