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Application of multi-way data analysis on excitation–emission spectra for plant identification

Safwan M. Obeidat [a], Tzach Glasser [b], Serge Y. Landau [b], Dean M. Anderson [c], Gary D. Rayson [a,*]

[a] *Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, Box 30001, NM 88003, USA*
[b] *Jornada Experimental Range, USDA, Las Cruces, Box 30003 MSC JER, NM 88003, USA*
[c] *Department of Natural Resources and Agronomy, Institute of Plant Science, Agriculture Research Organization, The Volcani Centre, Bet Dagan 50250, Israel*

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Abstract

The ability to distinguish among diets fed to Damascus goats using excitation–emission luminescence spectra was investigated. These diets consisted of *Medicago sativa* L. (alfalfa), *Trifolium* spp. (clover), *Pistacia lentiscus*, *Phyllirea latifolia* and *Pinus brutia*. The three-dimensional luminescence response surface from phosphate buffered saline (PBS) extracts of each material was analyzed using muti-way analysis chemometric tools (MPCA) and parallel factor analysis (PARAFAC). Using three principal components, the spectra from each diet material were distinguished. Additionally, fecal samples from goats fed diets of either alfalfa or clover hays were investigated. The application of MPCA and PARAFAC to these samples using models derived from the pre-digested diet materials was strongly suggestive of the utility of similarly derive training samples for the elucidation of botanical diet composition for animals.

Keywords: Luminescence spectroscopy; Multi-way principal component analysis (MPCA); Parallel factor analysis (PARAFAC); Phosphate buffered saline plant extracts

1. Introduction

In the Mediterranean region, goats are very important for brush control and ecological management [1]. The need to differentiate among goat diets is indeed a necessary issue for controlling free-ranging goats [2,3].

Fluorescence spectroscopy shows promise as a rapid and accurate method for identifying plant materials [4–7]. Earlier studies [5,6] used chloroform as the extracting solvent. Unfortunately, this solvent revealed fluorophores throughout the visible region of the spectrum including red chlorophyll fluorescence [8]. However, blue fluorescence from leaf material of higher plants has been suggested to result from a complex mixture of at least three fluorescing components [9]. Lichtenthaler et al. [10] later indicated the phenolic epidermal compounds in leaves (including cafferic, ferulic and sinapic acids as well as chlorogenic acid and quinic acid) may contribute to fluorescence in the blue region of the visible spectrum [10]. Additionally green fluorescence has been attributed to the cell wall components berberine and quercetin [11], epidermal tissue [12] and mesophyll tissue [13]. Although blue and green fluorescence result from multiple components [10], their chemical origins and locations within plants, are yet to be fully understood [14]. It may, however, be possible to differentiate among plant materials without a detailed understanding of the molecular species responsible for the resulting fluorescence signatures [5,6].

Recent research by Danielson et al. [15] suggested phosphate buffered saline (PBS) solutions as suitable solvents for extracting non-chlorophyll fluorophores from plant material. The exclusion of chlorophylls enabled a reduction of the masking of blue and green fluorescence signatures. Other work in our laboratories has also demonstrated the utility of some chemometric tools (e.g., principal component analysis (PCA) and

* Corresponding author. Tel.: +1 505 646 5839; fax: +1 505 646 2649.
E-mail address: garayson@nmsu.edu (G.D. Rayson).

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multi-way PCA) for the qualitative processing of luminescence spectra from PBS plant extracts [3].

The aim of the present study was to investigate the ability to differentiate among five different goat diets using fluorescence excitation–emission matrix (EEM) spectra of PBS extract solutions by applying multi-way principal component analysis (MPCA). Additionally, an MPCA calibration model was constructed from diets containing each of two hay species (i.e., alfalfa and clover) in an attempt to identify feces collected from different goats fed each diet. The number of the possible fluorophores in each of the five diets was also investigated using parallel factor analysis (PARAFAC).

2. Experimental

2.1. Samples

Five pre-dried goat diet materials were investigated using spectral fluorescence analysis. These included two *hay* plant species, alfalfa (*Medicago sativa* L.) and clover (*Trifolium* spp.) and gree browse species, *Pistacia lentiscus* (*P. lentiscus*), *Phyllirea latifolia* (*P. latifolia*) and *Pinus brutia* (*P. brutia*). Feces samples of both alfalfa and clover were also investigated using the same technique. These materials were obtained from the Agricultural Research Organization of Israel. The composition of samples used in this study resulted from the actual diets of each of 12 Damascus yearling goats (mean weight of 38.5 ± 0.7 kg) at a feed study facility located south of the Carmel Ridge, Israel. This feed study involved feeding 10 goats alfalfa hay (samples 1–10 in Fig. 3) for 10 days and collecting the corresponding feces. Additionally, clover hay (samples 11–14 in Fig. 3) was fed to four goats for a period of 4 days with similar collection of the corresponding feces. Goat feces from both hay diets were similarly coded with the corresponding goat identification (Table 1). Other pure diet materials were added to this study to check the ability of the current technique to differentiate among different diet materials: and *P. latifolia* and *P. brutia* (15–17 and 18–20 in Fig. 3, respectively) and *P. lentiscus* (21–24 in Fig. 3). The facility consisted of roofed individual dirt-floor pens (1.7 m × 1.7 m) and a roofed collection corral where animals were placed between tests. Each pen was configured with a 15 l water bucket and a trough divided into two compartments for separation of the feed concentrate and the other materials presented to each animal. For more accurate intake measurements collection of residue of each material was facilitated by a shelf located beneath each trough. Diets were weighed and distributed once each morning during 12, 10-day tests. Fecal samples for alfalfa and clover were grab-collected each morning, midday and evening to minimize variance from digestive stages during each of the final five days [16,17].

Table 1
The identity of samples and goats

Sample number	Diet	Goat ID
1	Alfalfa	G
2	Alfalfa	A
3	Alfalfa	F
4	Alfalfa	I
5	Alfalfa	K (Missing)
6	Alfalfa	E
7	Alfalfa	D (Missing)
8	Alfalfa	H
9	Alfalfa	J
10	Alfalfa	B
11	Clover	C
12	Clover	B
13	Clover	M
14	Clover	F

2.1.1. Sample preparation

Diet and feces materials were initially ground to pass a 2 mm screen, placed in aluminum weighing boats and dried at 60° C for 24 h to constant mass. Three replicates of approximately 0.1500 g of each diet material as well as feces were weighed into separate borosilicate culture tubes (16 mm × 25 mm, Kimble Kontes, Vineland, NJ). The tubes were then sealed using Parafilm and stored at room temperature. All samples within each replicate sample set were randomized prior to analysis to minimize operator bias during data collection.

The phosphate buffered saline solution was autoclaved (35 min at 121 °C, 125 kPa) to minimize any microbial contamination. The solution pH was adjusted to 12.5 using 1.0 M NaOH (Mallinckrodt Chemical Works, Saint Louis, MO). Each 2 l volume of the PBS solution contained 0.263 g, NaN_3 (sodium azide, an additional microbial growth inhibitor), 1.422 g $NaHPO_4$, 3.801 Na_2HPO_4 (Alfa Aesar, Ward Hill, MA), 0.408 g KCl (Sigma, MO) and 13.567 g NaCl (J.T. Baker, Phillipsburg, NJ) dissolved in ultra-pure (18.0 MΩ) water.

Each replicate data set consisted of spectra from each of the five diet materials, fecal materials, three extraction solution blanks and a single solution consisting of a TiO_2 suspension. The spectrum of each blank was recorded at three times during the analysis of each replicate: the beginning, middle and end. The spectrum from the TiO_2 suspension solution was also collected three times during each replicate to account for any instrument drift. A total of 13 samples including the blanks and the TiO_2 solutions were run each day (a single replicate data set).

The incorporation of the light scattering suspension of TiO_2(s) provided a signal indicative of the wavelength-dependent intensity of the incident radiation. This enabled compensation for significant drifts in the output of the Xe-arc lamp excitation light source. Immediately following exposure of the TiO_2 sample, a blank spectrum was recorded.

A Lab Industries Repipet II (Barnstead/Thermolyne, Dubuque, IA), was calibrated to deliver 10.0 ml of extraction solution to each culture tube containing the diet and the fecal materials. Once filled, all 10 tubes were sealed with Parafilm and manually shaken. The tubes were shaken in an attempt to wet the "plug" of ground plant material that floated in each culture tube. These tubes were then agitated using an orbital shaker (VWR Model 98001;Albuquerque, New Mexico) at 100 RPM for 1 h. The culture tube openings were elevated slightly to minimize contact of the culture tube contents with the Parafilm. The culture tubes were also rotated 180°after 30 min to maximize

contact of the ground plant material with the PBS extraction solution.

The culture tubes were then centrifuged at 925 × for 25 min (Beckman Model TJ-6, Labx, Midland, ON, Canada). The liquid from each culture tube was subsequently decanted into a non-sterile 10.0 ml syringe (Allometrics Inc., Franklin Lakes, NJ) and filtered through a 0.2 μm non-sterile nylon filter (Millex, Bedford, MA). Approximately, 3 ml of filtrate was immediately collected in a 3.5 ml disposable acrylate fluorescence cell with a light path of 10 mm (Spectrocell, Oreland, PA). The cell was then capped (Spectrocell Teflon®, LDPE), placed within the fluorometer, and the resulting excitation–emission matrix collected for all samples including both the diet and the fecal materials. These comprised 1024 emission intensity measurements at each of 51 excitation wavelengths (370–580 nm in 4.2 nm increments).

2.2. The fluorometer

The fluorometer used in these studies [18–20], has been previously described by Mukherjee et al. [21]. Briefly, it consists of a 150 watt Xe-arc lamp (Oriel Model 6254, Newport Oriel Instruments, Stratford, CT) as an excitation source. Each wavelength of excitation was selected using an F/4, 1/8 m double monochromator with a bandwidth of 7 nm (CVI Model 120, CVI, Albuquerque, NM). Stray light was reduced using a band pass filter on the monochromator. Scattered light and fluorescence from the PBS filtrate were detected at 90 degrees to the incident radiation. The emitted radiation was imaged into the entrance slit of an F/4, 1/8 m imaging spectrometer (ISA Jobin Yvon, Edison, NJ), with a 200–700 nm range. A 1024-element intensified Reticon array (Model 1420, EG&G Princeton Applied Research, Trenton, NJ) detected the light at the image plane. The detection spectrometer had a 5 nm bandbass.

2.3. Data collection

Instrument control and data acquisition were accomplished using software developed using Lab View software (Version 7.0, National Instruments, Austin, TX) installed in a Gateway desk top PC equipped with a Pentium II processor. The spectral intensities are reported on a relative intensity scale and were blank corrected. Wavelength regions of the spectra were divided in to arbitrary spectral regions and designated as either "blue" (424–491 nm) or "green" (491–575 nm) [22].

3. Statistical analysis

3.1. Multi-way principal component analysis (MPCA)

Chemometric data analysis methods provide powerful tools to analyze multivariate data such as excitation–emission matrices obtained from fluorometry [23]. Principal component analysis and MPCA are multivariate statistical methods for analyzing data measured as a function of two or more parameters (multi-way data) [24].

MPCA is one of the most direct approaches for decomposing the EEM [25]. Statistically and mathematically, MPCA is very similar to PCA and involves the generation of a representation of the eigenvectors for the covariance or correlation matrix of the original measured variable data matrix. There may be as many eigenvectors as there are variables. Each principal component generated describes diminishing contributions of the variance among the measured variables [25].

Consider the measurement of intensity at each jth ($j = 1,\ldots, J$) emission wavelength for every kth excitation wavelength ($k = 1,\ldots, K$) corresponding to the ith sample ($i = 1,\ldots, I$). These data can be then be organized into a three-dimensional matrix $\boldsymbol{X}$ of dimension $I \times J \times K$. In MPCA, the unfolded matrix $\boldsymbol{X}$ is subsequently decomposed into a large two-dimensional matrix $\boldsymbol{X}$ (Fig. 1) followed by conventional PCA. Simply stated, MPCA is the summation of the product of score vectors ($\boldsymbol{t}_r$) and loading vectors ($\boldsymbol{P}_r$) plus a residual or error array ($\boldsymbol{E}$) which is minimized in a least squares sense.

$$\boldsymbol{X} = \sum_{r=1}^{R} \boldsymbol{t}_r \otimes \boldsymbol{P}_r + \boldsymbol{E} \quad (1)$$

Each element of score vectors ($\boldsymbol{t}_r$) corresponds to a particular data set. The loading vectors ($\boldsymbol{P}_r$) are then directions of a maximum variability and define the reduced dimension space (R).

In most cases, only few principal components can be used to express the maximum variability especially in data with a high degree of correlation ($R \gg \min(I, JK)$). The choice of R is made for the optimization of the systematic variability of the data and can be described by these few principal components. Additionally, the residual array ($\boldsymbol{E}$) is minimized according to the least squares sense [26].

3.2. Parallel factor analysis (PARAFAC)

The PARAFAC is another powerful multi-way data analysis tool that assumes a liner relationship between the excitation–emission wavelength pair. It was used to investigate the number of factors (i.e., fluorophores) responsible for features within each spectral signature.

Like MPCA, PARAFAC is an algorithm that decomposes multi-way dimensional arrays into a set of scores and loadings. Because PARAFAC is a constrained version of PCA, any data set that can be modeled by PARAFAC can also be modeled by PCA [27].

Mathematically, the PARAFAC algorithm decomposes the three-dimensions data matrix ($\boldsymbol{X}$) into a sum of triple product of vectors (components or factors) and an error matrix e (Eq. (2)). While for PCA, each component consists of one score vector and one loading vector, each component (factor) in PARAFAC consists of one score vector and two loading vectors. These components or factors are organized into spectral matrices (i.e., a–c). Each matrix represents a single dimension of the original data cube containing N factors (N is the smallest number of independent factors that can be used to efficiently describe the data

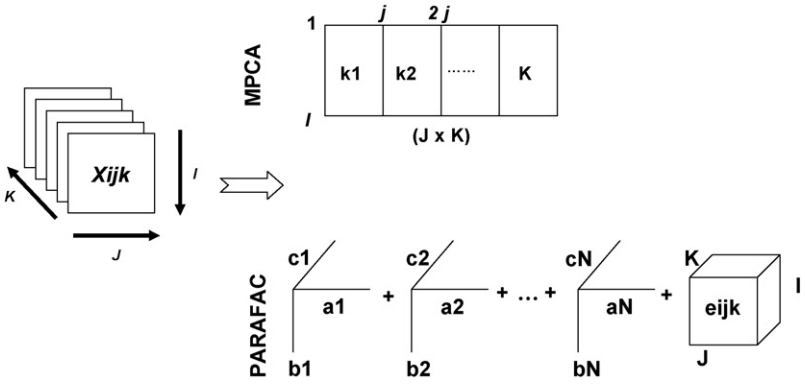

Fig. 1. Unfolding of three-way array $\boldsymbol{X}$ (I, J, K) into a two-way array in MPCA and its decomposition into a sum of vector or loading products, J and K. And a sum of triple product of vectors or loadings a–c, and error matrix $\boldsymbol{E}$ in PARAFAC.

variances) (Fig. 1) [27–29].

$$\boldsymbol{X}_{ijk} = \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} + e_{ijk} \quad (2)$$

In Eq. (2), $\boldsymbol{X}_{ijk}$ is a three-dimensional data set (i.e., fluorescence intensity of sample k at excitation wavelength i and emission wavelength j), N the unique spectral profiles found in the data cube, the n columns of matrix a are the predicted pure excitation of the n factor, the n columns of b are the predicted pure emission spectra of the n factor and the columns of c are the predicted pure spectral intensity profiles of n factors and e_{ijk} is the error matrix.

4. Results and discussion

No detectable degradation of the samples was observed over the 3-day data collection period. Additionally, the different PBS solution blanks were found not to differ statistically ($P = 0.912$). Therefore, the mean blank spectrum was subtracted from each sample spectra prior to analysis using MPCA and PARAFAC (PLS-Toolbox, Eigenvector Research, operated under MatLab 7.0.4, MathWorks, Natick, MA). The data were mean centered before applying MPCA. Prior to application of PARAFAC the data were weighted to account for scattering signals and truncated to contain only the regions of the spectra that had significant fluorescence information (i.e., $\lambda_{em} \geq \lambda_{ex}$).

4.1. Diet samples

Fig. 2 shows a typical excitation–emission luminescence spectrum for a PBS extract of an alfalfa hay sample. The broad lines with slopes of approximately 1.0, and 2.0 correspond to the first and second order diffraction of the incident radiation (λ(emission) = λ(excitation)), respectively, that displays blooming into adjacent pixels. It is, therefore, the region located above this first order Rayleigh scattered radiation that is of primary analytical utility (i.e., λ(emission) $\geq$ λ(excitation)). Because the Rayleigh scattering features are diagonal lines, studying the region above this scattering results in the loss of that portion of the signal exhibiting an overlap with the Rayleigh scattering [29].

Weighting of the data within each EEM by replacing the values of the Rayleigh scattered signal pixels with zeros has been proposed as an approach to address this problem [30]. This was accomplished by multiplying the data matrix by a weighting matrix that has the same size as the data matrix in which the regions of the matrix corresponding to the Rayleigh scattering are given values of zero and values of one for the rest of the matrix. Weighting the data for PARAFAC application has been proposed as an approach to address the problem associated with the presence of the Rayleigh scattering. For the application of PARAFAC, the data were subjected to both a discrete weighting strategy and truncation of the data sets to include only those spectral regions containing useful signal [29].

The spectral signatures for each material were each recorded several times in accordance to the actual feed study for goats described elsewhere [3]. A three-dimension data matrix containing the EEM for each of the five materials was generated (λ_{ex},λ_{em}, material). These were subsequently processed using both MPCA and weighted PARAFAC. Two-dimensional MPCA models using either the first and second or the first and third principal components were only able to separate the five diet

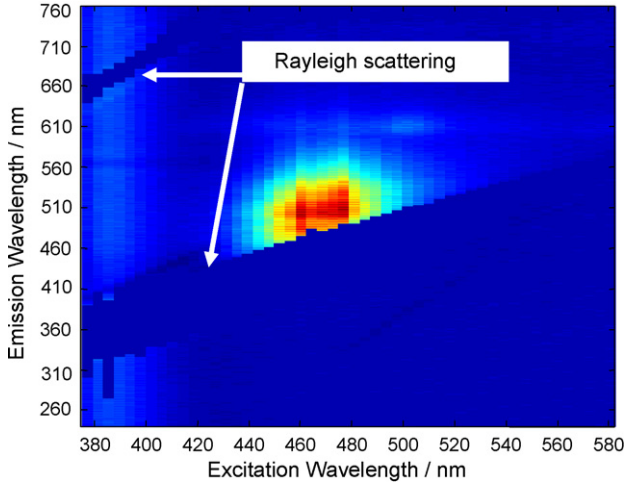

Fig. 2. Contour plot representation of excitation–emission matrix (EEM) recorded from the PBS extract of a sample of *Medicago sativa* L. (alfalfa) showing both first and second order diffraction of the Rayleigh scattered incident radiation.

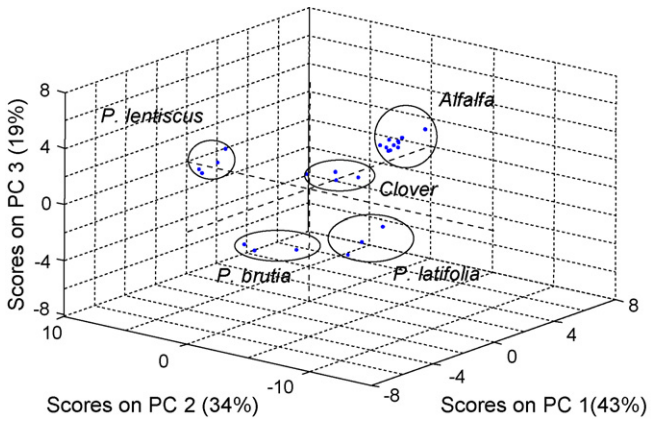

Fig. 3. Three-dimensional MPCA scores plot for each of the materials analyzed. Each point represents a single spectrum acquired for each replicate from each diet material, *Medicago sativa* (alfalfa) hay (samples 1–10), *Trifolium spp.* (clover) hay (samples 11–14), *Phyllirea latifolia* (samples 15–17) *Pinus brutia* (samples 18–20), *Pistacia lentiscus* (samples 21–24). Circles around each cluster are for clarity and do not represent confidence limits.

materials into only four clusters, leaving the *P. brutia* samples distributed among the other clusters. However, application of a three-dimensional MPCA model (Fig. 3) enabled more than 95% of the total variation in the original data matrix to be accounted for using three principal components. Projection of the scores for each sample spectrum yielded five separate, very well resolved clusters (Fig. 3). Each of these five clusters represents one of the diet materials. The centers of each of the five clusters were calculated and the standard deviations of each point in each cluster from its pre-calculated centers were also calculated. The averaged standard deviation for the clusters of alfalfa, clover, *P. lentiscus*, *P. latifolia* and *P. brutia* were 2.7, 1.3, 4.2, 3.0 and 2.9, respectively. Despite the above variations, which might affect the confidence limits of detection for the individual species, discrimination among the plants species used is readily apparent. These results strongly suggest the ability of this technique to discriminate among the five diet materials used in this study.

For the purpose of identifying the possible number of fluorophores in each diet material and their corresponding excitation–emission profiles weighted PARAFAC was also applied on each of these five separate diet materials to investigate the number of fluorophores associated in each of the five plants. The number of factors for each model was determined using both the core consistency test along with visual inspection [28]. PARAFAC application was employed for the truncated data matrices that have significant fluorescence signals. The studied regions of the data matrices for all the samples included excitation and emission boundaries of 415–565 nm and 450–710 nm, respectively.

One or two factors were found among the five diet materials. Both PARAFAC excitation and emission profiles for the five diet materials were investigated. Fig. 4 shows the excitation profiles for each of the five diet materials. For samples such as alfalfa, *P. latifolia* and *P. lentiscus* (Fig. 4a–c, respectively), one factor was identified. This suggests only one fluorophore in these materials is responsible for the measured fluorescence. Fig. 6a–c show the corresponding emission profiles for each factor. In alfalfa, the excitation–emission profile for the factor lies at an excitation wavelength of 465 nm (Fig. 4a) and an emission wavelength of 520 and 620 nm (Fig. 5a). However, the factor revealed for *P. latifolia* was observed to have different excitation–emission profile with excitation and emission wavelength maxima at 480 (Fig. 4b) and 550 nm (Fig. 5b), respectively. The *P. lentiscus* factor exhibited excitation–emission wavelengths of 480 and 570 nm (Figs. 5c and 6c, respectively). This result is consistent with the MPCA analysis above. Although only one factor was observed in alfalfa, *P. latifolia* and *P. lentiscus*, these factors revealed different excitation–emission profiles, thus enabling them to be distinguished. This was also revealed in separate clusters of alfalfa, *P. latifolia* and *P. lentiscus* in the space of the MPCA model (Fig. 2).

Two factors were found in the remaining materials (Figs. 5 and 6). However, the two factors in each of these samples have unique excitation–emission profiles. Fig. 4d shows the excitation profile of the two factors (blue and green) found in *P. brutia*. The blue factor has an excitation maximum of 420 nm with corresponding emission maxima at 475 and 625 nm (Fig. 5d). Conversely, the green factor displayed an excitation maximum at 480 nm (Fig. 4d) and emission maximum wavelengths at 505 and 625 nm (Fig. 5d). The clover hay sample also revealed two factors. Fig. 4e shows the excitation profile for these factors. The green has an excitation peak at 450 nm and emission maxima at 490 and 610 nm (Fig. 5e). Comparatively, the blue factor showed a peak excitation wavelength of 490 nm (Fig. 4e) and emission wavelength maxima at 530 and 610 nm (Fig. 5e). Again this is consistent with the MPCA analysis. Significant differences in the spectral signatures in these samples (Fig. 3) were indicated through the clustering of each diet material samples in a separated cluster.

Table 2 summarizes the factor number for each of the studied samples a long with the excitation–emission profile for each factor.

4.2. Fecal samples

Fecal samples from animals fed diets consisting of each of the two forvs (alfalfa and clover hay) were similarly analyzed. The

Table 2
PARAFAC factors for each material with the locations of maxima in each resulting excitation and emission profile

Species	Number of factors	Excitation wavelength (nm)	Emission wavelength (nm)
Alfalfa hay	1	465	520, 620
Phyllirea latifolia	1	480	550
Pistacia lentiscus	1	480	570
Pinus brutia	2	420	475
			625
		480	505
			625
Clover hay	2	450	490
			610
		490	530
			610

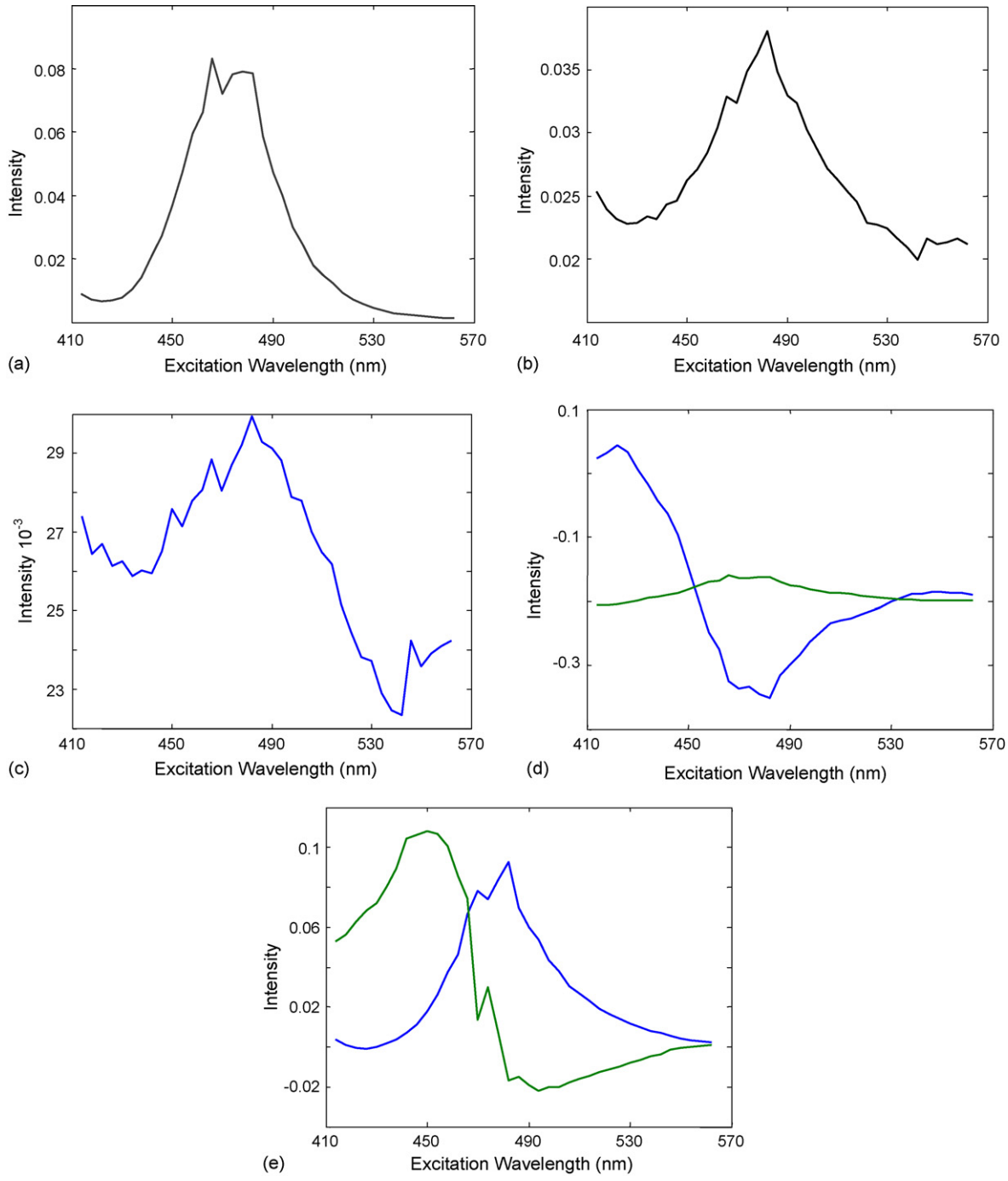

Fig. 4. Excitation profiles of PARAFAC model for diet samples of (a) *Medicago sativa* (alfalfa), (b) *Phyllicea latifolia*, (c) *Pistacia lentiscus*, (d) *Pinus brutia* and (e) *Trifolium spp.* (cover) hay.

goal of this study was to provide preliminary data regarding the potential application of chemometric models (i.e., MPCA and PARAFAC) derived from diet materials and fecal material samples for the determination of botanical diet materials a MPCA model was therefore constructed using PBS extracts of samples of each of two diet materials species used in Fig. 3, alfalfa and *clover hay* (circles in Fig. 6). (Availability of corresponding fecal samples from animals fed only the single plant species limited these studies to these two-diet materials.) Then extracts from feces collected from each of individual goats were applied to the above MPCA calibration model (triangles in Fig. 6). Readily apparent are the segregation of spectra based on the plant species for both diet and fecal samples (i.e., alfalfa hay versus clover hay) and the similarities in the projection of spectra from the pre- and post-digested samples of these same plants. Not surprisingly, the greatest distribution of projected points is observed for spectra from fecal samples collected from the different animals fed these diets. The cluster centers for both diet materials, alfalfa and clover in this model were calculated as well as cluster centers for the corresponding fecal samples. Then the maha-

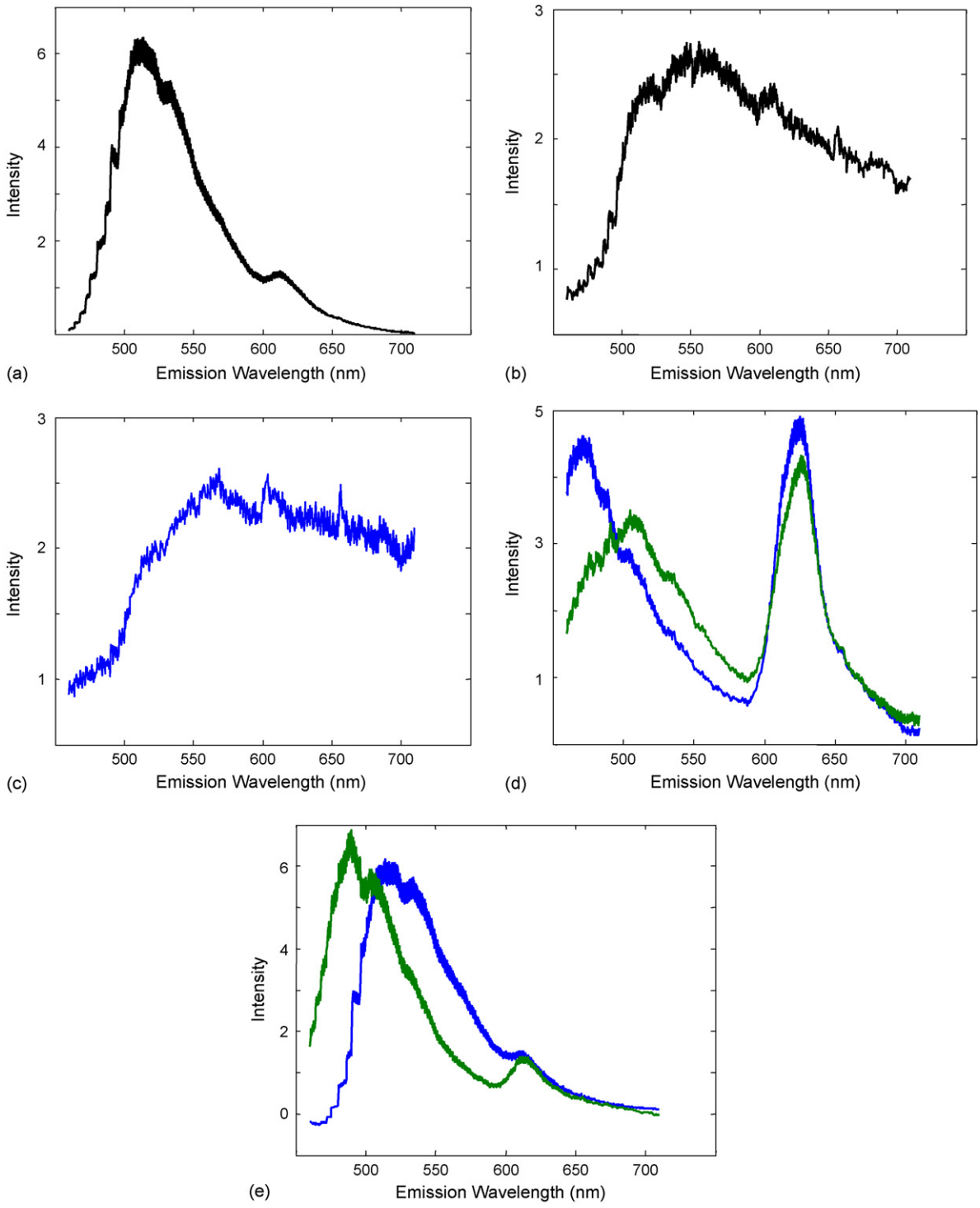

Fig. 5. Emission profiles of PARAFAC model for diet samples of (a) *Medicago sativa* (alfalfa), (b) *Phyllirea latifolia*, (c) *Pistacia lentiscus*, (d) *Pinus brutia* and (e) *Triffolium spp.* (clover) hay.

lanobis distance between the centers of diet materials clusters and the centers of the corresponding fecal samples clusters were calculated. It was determined that the distance between the diet material center of alfalfa and its corresponding fecal center was 13 units. Similarly the distance between the clover diet material center and the corresponding clover fecal samples center was five units. The distance between the centers of alfalfa diet materials and the fecal samples of clover was similarly calculated and found to equal 23 units. Additionally, the distance between each of the centers of clover diet material and the fecal samples from alfalfa was 18 units. This supports the visual interpretation from Fig. 6 which suggests the clustering of the same species together for both diet and and fecal materials, independent of the animal involved.

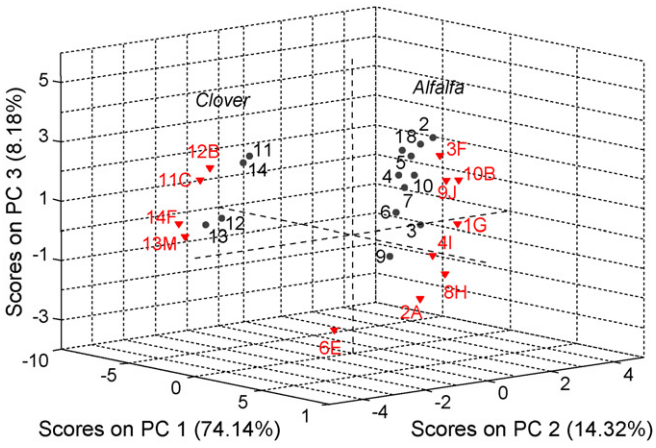

Fig. 6. Three-dimensional MPCA scores plot for a model generated by spectra from diet samples of *Medicago sativa* (alfalfa) hay (1–10) and *Trifolium spp.* (clover) hay (11–14) (●) and the application of that same model to spectra from fecal samples (▲) collected from individual goats (letter code) fed those same diets.

To further investigate the source of variance among these samples, and to investigate the possible number of fluorophores responsible for the spectral feature of each species in this research PARAFAC was applied to spectra derived from the feces of only animals fed each of these materials (i.e., alfalfa and clover hay). Comparison of the resulting PARAFAC profiles (excitation, Fig. 7 and emission, Fig. 8) for the pre- (Figs. 4 and 5) and the post-digested (Figs. 7 and 8) forms of alfalfa and clover hay reveal good consistency.

Fig. 7a, shows that only one PARAFAC factor is responsible for the spectrum in the post-digested form of alfalfa. The excitation profile for the alfalfa fecal samples indicated a maximum at 450 nm (Fig. 7a) while that from the pre-digested form displayed a maximum at 465 nm (Fig. 4a). In contrast, the corresponding emission profiles for both forms of alfalfa diet samples were very similar with maxima at 520 and 610 nm (Figs. 6e and 8b).

PARAFAC revealed each of two factors for the clover hay (blue and green curves) in the spectra for both pre- and post-digested samples. The excitation profile for the fecal samples of the clover hay (Fig. 8a) displayed a maximum at 445 nm compared to 450 nm arising from a similar analysis of pre-digested

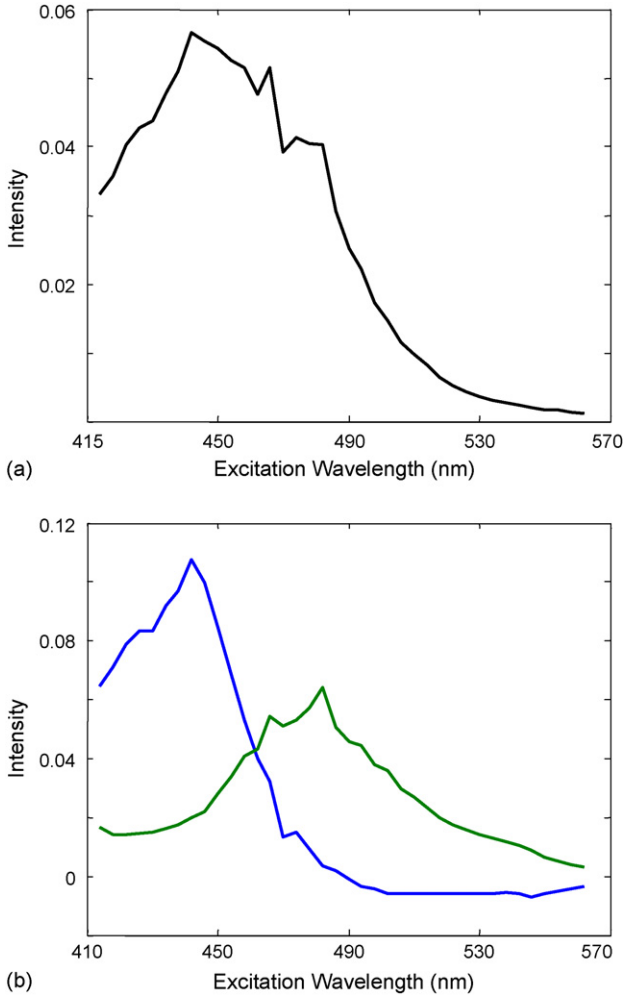

Fig. 7. Loadings from the PARAFAC model (excitation spectra) applied to fecal samples corresponding to diets consisting of (a) *Medicago sativa* (alfalfa) hay and (b) *Trifolium spp.* (clover) hay.

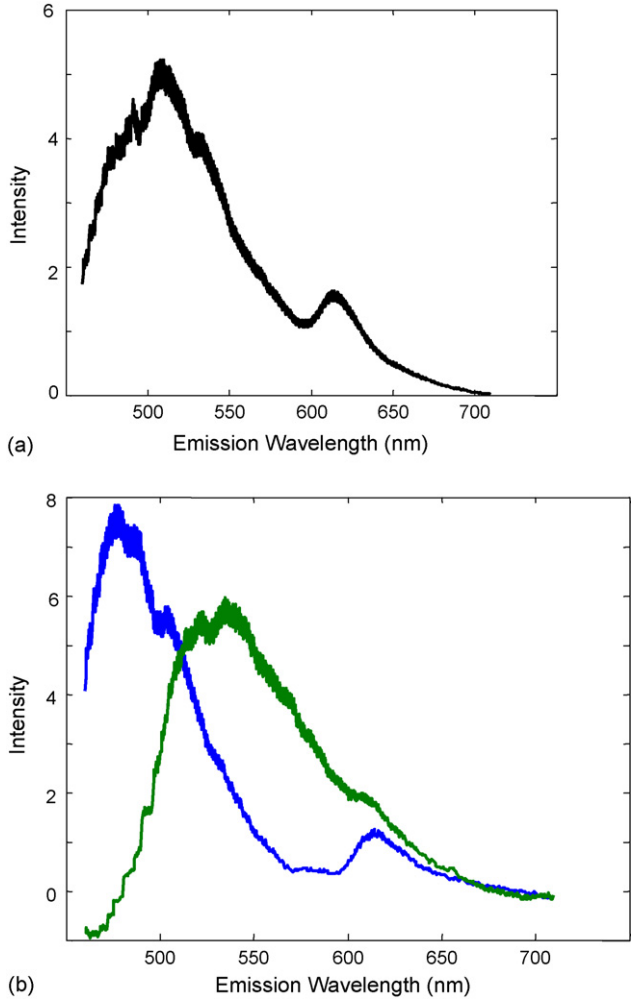

Fig. 8. Loadings from the PARAFAC model (emission spectra) applied to fecal samples corresponding to diets consisting of (a) *Medicago sativa* (alfalfa) hay and (b) *Trifolium spp.* (clover) hay.

diet samples (Fig. 4e). The emission profile for the same factor (blue) indicated maxima at 480 and 610 nm (Fig. 8b) compared to 490 and 610 nm (Fig. 5e). The second factor (green) revealed a peak at 490 nm in the excitation profile for the fecal extract sample (Fig. 8a) with a maxima in the corresponding emission profiles at 550 and 610 nm (Fig. 8b). This is in comparison with the corresponding excitation (Fig. 4e) and emission (Fig. 5e) profiles for the pre-digested diet samples that exhibited maxima at 490 or 530 and 610 nm, respectively. The similarity and differences in these extracted spectra for the respective diet and fecal samples may contribute to the observed projections of the luminescence spectra using MPCA.

5. Conclusion

Three-dimensional luminescence spectra from PBS extracts of alfalfa and clover hay, and browse samples from the plants *P. lentiscus*, *P. latifolia* and *P. brutia* have been shown to enable material identification. The application of MPCA enables a qualitative identification of animal diet materials. Although the molecular species responsible for the observed spectral signatures is currently unknown, statistical models using PARAFAC suggest the number of a possible fluorophores to vary between (alfalfa hay, *P. lentiscus* and *P. brutia*) and (*P. latifolia*, clover hay). The excitation and emission profiles for the suggested fluorophres detected by PARAFAC are unique. This indicates that the chemical source behind the observed fluorescence is not the same among the current samples. However, in this research suggests that digestion may not affect the fluorescence spectral signatures significantly. This is clear after applying both MPCA and PARAFAC. The fecal samples continued to cluster very close to the diet material samples in the MPCA model that was constructed from the diet materials only. This supports the hypothesis that the spectral signatures for pre- and post-digested samples (used in this study) were similar. The application of these spectroscopic and statistical tools to fecal samples suggests the possible use of model learning sets derived from mixtures of plant or diet materials for the identification of botanical comparition of animal diets. This aspect of the work is the focus of ongoing research.

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