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Discernment of Lint Trash in Raw Cotton Using Multivariate Analysis of Excitation-Emission Luminescence Spectra

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Abstract

Excitation-Emission luminescence spectra of basic (pH 12.5) phosphate buffer solution extracts were used to distinguish among botanical components of trash within seed cotton. All components were separated from whole plants removed from a field in southern New Mexico. Unfolded Principal Component Analysis (U-PCA) was applied to Rayleigh-corrected extract spectra from each plant component. This enabled qualitative distinction of seeds, stems, bracts and leaves as trash material. Sensitivity of U-PCA models to sample replicate selection was also evaluated. This revealed significant sample dependence for U-PCA models using spectra from stems of both Pima and upland cotton varieties. Dependencies were also observed for seeds, burrs, walls, and fiber from Pima cotton. Spectra from fiber and seed samples of upland cotton also exhibited significant sample-selection sensitivity on U-PCA models. Discernment of lint trash components is therefore possible using three-dimensional luminescence spectra. Application of parallel factor analysis (PARAFAC) further revealed three spectral factors enabling trash identification. Elucidated excitation and emission spectra of each factor exhibited excitation wavelengths of maximum intensity (295, 320, and 400 nm) and respective emission wavelengths (348, 435, and 473 nm). These wavelengths suggest the presence of 1) tryptophan-containing proteins or polypeptides, 2) NADH, pyridoxic acid, or pyridoxic acid-5'-phosphate, and 3) 6,7-dihydoxy or 7-hydroxy-6-methoxy coumarins as the discriminating components for each respective factor, thus enabling an improved understanding of observed spectral differences among lint trash materials.

Introduction

Machine harvested seed cotton (cottonseed with fiber still attached) contains significant amounts of foreign matter consisting of seeds, soil particles, and other parts of the cotton plant. All cotton in the US is mechanically harvested by either spindle-pickers or strippers. Spindle harvested seed cotton typically requires approximately 1400 pounds of seed cotton to make a 480 pound bale of ginned cotton (217.7 kg) that can yield 75-200 pounds (34.0-90.7 kg) of trash [1] with the remainder being cottonseed. Cotton strippers harvest more trash with the seed cotton so stripped seed cotton can require up to approximately 2200 pounds (997.9 kg) of seed cotton for a 480 pound (217.7 kg) bale of ginned cotton. The increased weight for stripped cotton comes from up to 600 additional pounds (272.2 kg) of trash or foreign material [1]. Specifically, this picked or stripped foreign material includes soil particles, leaf fragments, immature seeds, stems, or burr fragments [2,3]. Failure to fully remove these materials can significantly degrade resulting fiber quality for textile processing Proper identification of the source of remaining foreign material in ginned cotton bales could help to change the ginning process to improve trash removal during ginning and fiber cleaning. Further reduction of trash levels in ginned cotton would improve the value of ginned cotton for textile feedstock.

Harvesting and ginning each contribute to non-fiber particle size reduction. This limits the utility of visual inspection, image analysis, or gravimetric methods for accurate identification of these materials [4]. Consequently, there exists a need for alternate methods to identify both trash type and source. Such evolving methods include those based on either the size and/or shape of trash particles or utilize spectroscopic tools [5].

Predominate techniques based on size and shape used currently includes: 1) High Volume Instrumentation (HVI) (Uster Technologies, Inc., Knoxville, TN); 2) The Advanced Fiber Information System (AFIS) (Uster Technologies, Inc., Knoxville, TN); 3) The Shirley Analyzer; and 4) The Cotton Trash Identification System (CTIS) [6]. Briefly, HVI uses particle size to generate trash component frequency distributions employing a scanning video camera. Comparatively, AFIS segregates dust and trash based on equivalent diameter criteria (i.e. 50-500 μm and > 500 μm, respectively) using an optical sensor. Alternatively, the Shirley Analyzer employs destructive gravimetric measurements to determine total trash in a given sample [7]. Unfortunately, many of these methods do not facilitate categorization of detected trash sources (i.e. leaves, stems, etc.) [8].

CTIS uses high resolution image analysis to distinguish trash and dust particles as small as 0.005 mm^2 (i.e. equivalent diameter of 40 μm) [9]. It has shown promise in identifying trash sources [10], but is limited by its application to small portions of the heterogeneous raw seed cotton material. Consequently, there

remains a need for trash source identification of larger seed cotton samples (e.g. bracts and leaves).

Spectroscopic methods for trash identification include the application of Near Infrared Reflectance (NIR), mid (or normal) IR absorption (MIR) [11] and molecular fluorescence [4]. NIR involves the comparison of measures reflectance in the wavelength range of 0.700 to 2.0 μm to spectra corresponding to harmonics and overtones of molecular vibration frequencies. Spectral overlap of features from various chemical functionalities requires the use of chemometric statistical tools (e.g., PCA, PLS). The advantage of this approach is its ability to sample areas of several centimeters very quickly e.g., < 5s) [12]. Unfortunately, it is a single variable response (i.e. reflectance as a function of the wavelength of incident light) and is susceptible to unknown sample composition variance [11]. MIR is based on absorption of light corresponding to fundamental modes of molecular vibrations within the sample. While more easily interpreted then NIR spectra, it provides very limited discrimination in chemically complex samples (e.g., cotton fibers) [13,14]. (Non-lint components of cotton differ significantly in spectral characteristics and no one NIR measurement could indicate collective content much less differentiate between non-lint components. Also proper uniform lighting of samples was difficul to obtain (Taylor). Using NIR band ratios also did not correlate well with detecting non-lint content of cotton (Thomasson and Shearer). Gamble and coworkers [4] have also reported the use of fluorescence from organic solvent extracts (i.e. dimethyl sulfoxide, DMSO). Also Himmelsbach et al. [15] reported potential contributions of anthocyanin or proantocyanidin compounds to measured fluorescence from cotton seed coats. Further application of molecular fluorescence is the subject of the present work.

Recent work in our laboratory has demonstrated the ability to distinguish among samples of different plant species using fluorescence spectra of aqueous extracts [16-19]. Multivariate analysis of excitation-emission spectra from phosphate buffered saline (PBS) plant extracts enabled segregation of both six different plant species [17] and different animal diets [19]. This experience led us to investigate the ability of this technique to identify trash components found within gram-sized samples of raw, unprocessed (i.e. seed) cotton.

Materials and Methods

Entire, mature cotton plants were manually collected from the Leyendecker Plant Science Farm, New Mexico State University, Las Cruces, NM, USA. Plants of both Pima and upland cotton species were collected. Each plant contained several complete bolls. Various plant components were manually separated from intact bolls while wearing latex gloves to minimize any potential sample-handling contamination. These components included stems, leaves and petioles (as a single component), bracts, seeds, fibers, the outer burr, the burr mid-wall, and the open burr. These are each illustrated in Figure 1 and described in Table 1.

Triplicate samples of each material from each cotton species (e.g., Pima and upland) were prepared by removing all other foreign objects. The air-dried materials were cut into small pieces and then ground using a mortar and pestle. Triplicate samples were treated with 8.0-10.0 mL of a pH 12.5 PBS solution [18,19]. Each of two separate data sets was generated several months apart in time to evaluate the effect of time on potential changes in the plant materials. As indicated above, similar fluorescence studies were described previously by Gamble, *et al.* using DMSO [4]. They indicated extraction of some components (e.g., chlorophyll leaves) was problematic in their study. This interference was minimized in the present work by using a PBS extracting solution [17]. Use of basic conditions (i.e. pH 12.5) was also reported to enable greater discernment among materials [17]. Based on the successful use of these conditions (i.e. pH 12.5 PBS) to distinguish plant materials, this same extraction solution was used in the present study.

The PBS solution was prepared from 116 mM sodium chloride (NaCl), 2.7 mM potassium chloride (KCl), 5 mM disodium phosphate ($Na_2HPO_4.H_2O$), 5 mM trisodium phosphate (Na_3PO_4) and 2.03 mM sodium azide (NaN_3) in distilled water [17]. The sodium azide inhibits microbial growth under aerobic conditions [17]. The solution pH was iteratively adjusted to 12.5 by drop-wise addition of either concentrated HCl (12 M) or a saturated NaOH solution. Solution pH was monitored using an Orion pH electrode (model 710A). Although all solutions were autoclaved prior to their use it was necessary to further minimize the impact of such growth by reducing sample processing time (initial solution contact to spectral data collection) to less than four hours.

Following addition of the PBS solution, samples were agitated for about one hour using a rocker. The resulting colorless or green supernatant solution was then transferred and centrifuged for 2 minutes at 900 rpm using a bench top centrifuge (International Equipment Co., model CL, Needham, MA). The supernatant was transferred to a 1 cm cuvette and the fluorescence spectrum recorded (Cary Eclipse, Varian, Palo Alto, CA). This protocol was sufficien to separate all tissues from the plant material. The excitation wavelength, λ_{exc}, was scanned from 270 nm to 450 nm in 5 nm increments. Each corresponding emission spectrum, λ_{em}, was also recorded from 300 nm to 600 nm in 5 nm increments. The band pass of the instrument (excitation and emission) was adjusted to 2.5 nm to insure the

Table 1: List of botanical components of cotton plants investigated within the present study.

Botanical components of cotton plant	Description
Stem	Cylindrical aerial above ground part of the plant that supports vegetative and reproductive organs.
Leaf and petioles	The specialized green organs that carry out the process of photosynthesis. Petioles refer to the stalk that attaches the leaf to the stem.
Bracts	Modified leaf tissue associated with the plant's flower.
Seeds	Fertilized ovule that contains an embryo.
Fiber	White tube-like structures that covers the seed that comprises the boll.
Open bracts	Capsules that bear the seeds.
Open bracts without cotton	The opened burr without bolls and fiber.
Outer burr	Outer cover of the burr of the cotton capsule.
Midwall of burr	Central cover of the burr, when opened.
Open burr	Outer burr and midwall of the burr taken together.

independence of each intensity measurement within the resulting fluorescence response surface. Representative excitation-emission spectra of extracts for each material shown in Figure 1 are provided in Figure 2. The resulting spectra (a 37 by 61 matrix for each sample) were processed using PLS-Toolbox 3.5 (Eigenvector Research, Wenatchee, WA) operated within MATLAB version 7.1 (Mathworks, Lowell, MA) as described below. These spectra differed significantly from those discussed by Gamble et al. [4] which utilized only a single excitation wavelength, i.e. 300 nm.

Data Analysis

Principal component analysis (PCA) involves reduction of variables within a data set to a few orthogonal components [20]. Each component is determined through calculation of eigen values within the covariance matrix for a data set. The first of these principal components explains the largest amount of variance within the original data. The distance each measured response is from the corresponding eigenvector is the score for that component's measurement. Similarly, additional orthogonal eigenvectors are identified for decreasing percentages of the data variance and the respective score values computed.

When the measured responses are functions of variables in two or more dimensions (e.g., fluorescence as a function of both λ_{exc} and λ_{emis}), the resulting three-dimensional data matrix (i, j, k, samples for each combination of λ_{exc} and λ_{emis}) can then be unfolded to a two-dimensional matrix of dimensions i (samples) and j×k ($\lambda_{exc} \times \lambda_{emis}$). Subsequent application of PCA to this unfolded matrix (i.e. unfolded PCA or multiway PCA, U-PCA or MPCA, respectively) then results in a similar variable reduction to a few principal components and the corresponding score values.

Cluster analysis enables the grouping of samples based on the proximity of each sample projection within a score space derived from the number of principal components used within the PCA model. These distances are weighted by the associated covariance (i.e. the Mahalonabus distance) to each neighboring point (i.e. k-nearest neighbor).

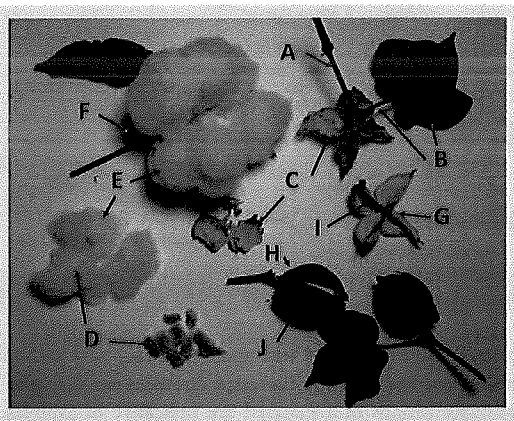

Figure 1: Different parts of a cotton plant recognized in the ginning process. A = Stem; B = Leaf and petiole; C = Bract; D = Seeds; E = Fiber; F = Open Bract; G = Open burr without cotton; H = Outer burr; I = Midwall of burr; J = Open burr.

Parallel factor analysis, PARAFAC, involves trilinear decomposition of multidimensional data sets. The data matrix is decomposed into three matrices comprised of vectors for each factor corresponding to the scores and each of two loadings according to the following equation [20-24].

$$X_{jki} = \sum_{n=1}^{N} a_{jn} b_{kn} c_{in} + e_{jki}$$

Where X_{jki} represents a data matrix with dimensions as discussed above (i.e. fluor scence intensity of sample i at excitation wavelength j and excitation wavelength k), N is the number of factors found to describe the original data set, the n columns of matrix a are the predicted pure excitation of the nth factor, the n columns of b are the predicted pure emission spectra of the n factor and columns of c are the predicted pure spectral intensity profiles (quantities) of each n factor. The component e_{kji} is the associated error matrix. (Note: Indexing variables have been selected to be consistent with those used in the previous discussion of U-PCA)

To minimize the impact of sample-independent signals and background noise on subsequent data analysis, Rayleigh scattering signals were removed from the resulting excitation-emission spectra. This was accomplished by applying a weighting factor of zero to data points corresponding to $\lambda_{emission} = \lambda_{excitation} \pm 5$ nm as proposed by Jiji and Booksh [24]. Anti-Stokes portions of the data set were also zeroed to reduce contribution from this nose-filled spectral region to computed models (Figure 2).

Triplicate sample excitation-emission fluorescence spectra from each of the materials were processed using multivariate analysis (Unfolded Principal Component Analysis, U-PCA, and Parallel Factor Analysis, PARAFAC). Models for each cotton species involving all combinations of the replicate sample spectra revealed three principal components (PCs) accounting for >89% and >91% of the variance (65.18, 19.14, and 5.35% from Pima and 62.97, 25.39, and 2.84% from upland cotton, respectively). Figure 3 shows the resulting scores for each material listed in Table 1 for Pima (A) and upland (B) cotton plants. Comparatively, Figure 4 shows the results of cluster analysis applied to these results using k-nearest neighbor criteria based on the respective Mahalonabus distances between the score-space projections of each spectrum for the respective species [20,23].

Results and Discussion

Principal component analysis

Rayleigh and anti-Stokes corrected excitation emission luminescence spectra for all spectra were combined into a single 33 by 37 by 61 matrix. Following unfolding of the third dimension (i.e. excitation wavelength), the resulting 33 by 2257 matrix was subjected to cluster analysis subsequent to PCA (Figure 4). A total of 3 components were found to account for 89.67% and 91.20% of the variance in the data for Pima and upland cotton samples, respectively. The criteria of k-nearest neighbor was applied to the Mahalonabus distances between the projected points in 3 dimensional score space. Figure 4 shows the resulting dendograms for Pima and upland cotton materials (Figures 4A and B, respectively).

From this analysis of all 54 samples (including replicates), bracts and burrs were found to result in significantly different spectral signatures. However, many of the materials were not easily

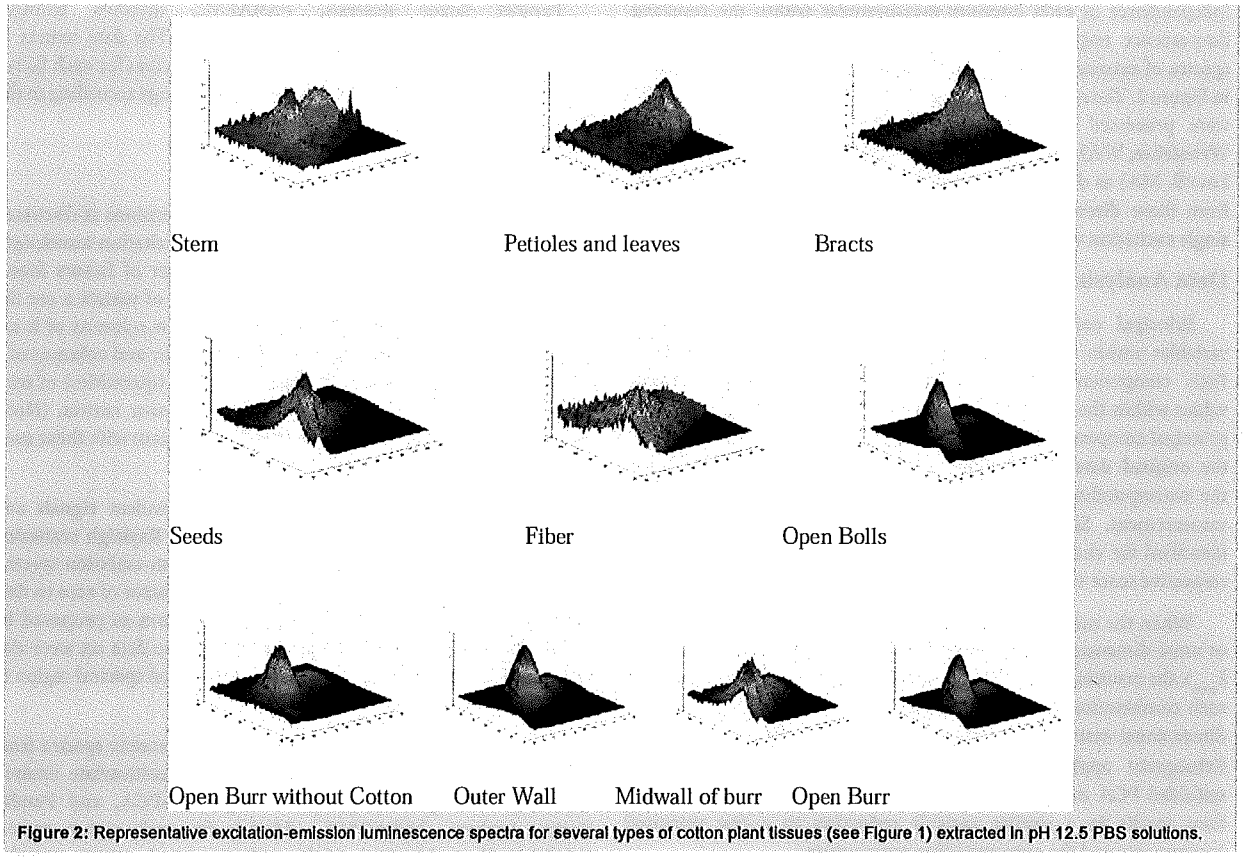

Figure 2: Representative excitation-emission luminescence spectra for several types of cotton plant tissues (see Figure 1) extracted in pH 12.5 PBS solutions.

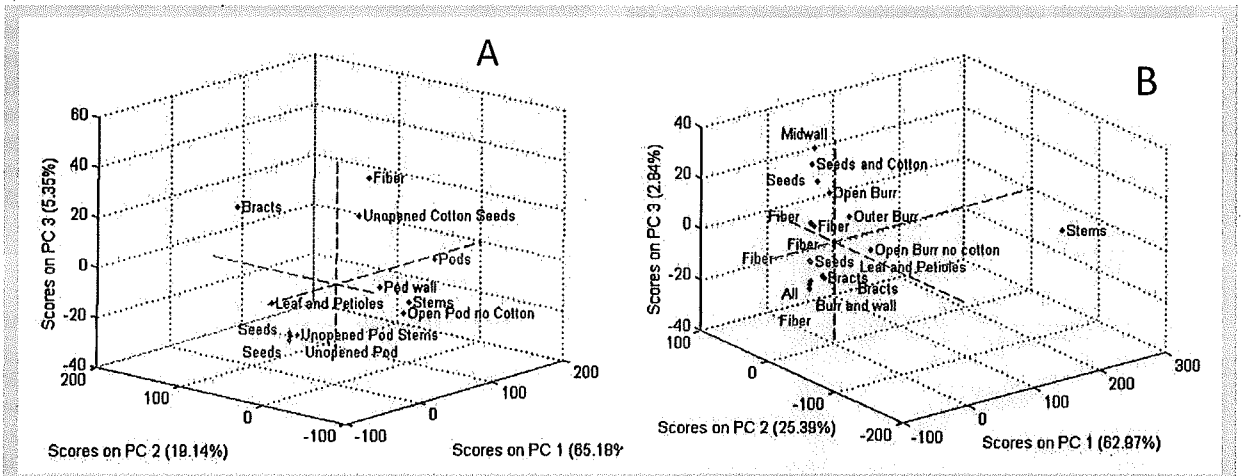

Figure 3: Score plots from U-PCA of excitation-emission luminescence spectra obtained from pH 12.5 PBS solutions of Pima (A) and upland (B) cotton plants.

differentiated with that model. In an effort to test the potential of sample-to-sample variation in resulting models and test the validity of those models, a series of U-PCA models were constructed using single replicates of each material (Figure 1). Spectra from each additional replicate were then applied to the model and the Cartesian distance from each test sample to its respective modeled sample was calculated. This procedure was applied to each test set/model set combination. The resulting Cartesian distances are shown in Figure 5. Multiple listings of a material are indicative of samples of the same plant component with subsequent replicate extract analyses. This enable distinction between prediction errors associated with model validation and those resulting from variability in material

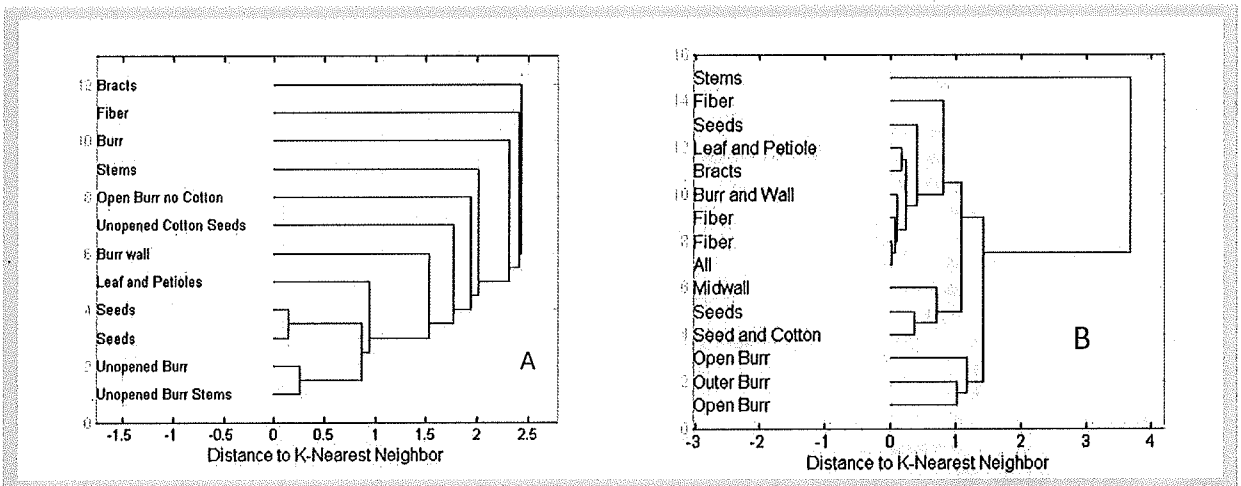

Figure 4: A dendogram from the application of cluster analysis of excitation-emission luminescence spectra from pH 12.5 PBS extracts of cotton plant parts (Figure 1) using k-nearest neighbor and Mahalonabus distances for Pima (A) and upland (B) cotton materials.

heterogeneities. Smaller distances indicate less sample-to-sample variability for the same material (e.g., many upland cotton materials in Figure 5B). Conversely, larger distances suggest greater sample dependence on the resulting model. From this analysis, stems and fiber samples from Pima cotton and stem and open bract samples from upland cotton were found to exhibit greater variability among replicates. These conclusions are further supported by the results shown in Figures 3 and 4.

Variations in luminescence spectra for replicate samples of the same material may be suggestive of the heterogeneity of the original materials. Extracts of cellulose-based cotton fibers would not be predicted to yield extracts exhibiting significant photoluminescence and would be predicted to be very homogeneous in composition. However, samples of both Pima and upland cotton fibers yielded sample-dependent luminescent solutions. One explanation could be the presence of small pieces of trash material, not visible to the naked eye. Such contaminates would then be expected to yield the corresponding spectral signatures of the particles within each sample collected and analyzed and exhibit potentially significant sample-dependent variability. This interpretation is consistent with the results shown. It should be noted that due to pretreatment of the spectral data (autoscaling and mean-centering), only a qualitative interpretation of the results of unfolded Principal Component Analysis is justified

Parallel Factor Analysis

In an effort to better understand the sources of these observed variations in luminescence signatures, the recorded excitation-emission spectra were further analyzed using PARAFAC. Application of PARAFAC to the entire sample set spectral signatures revealed the contribution of three factors to account for variability among the samples similarly to that determined using U-PCA. The resulting loadings are shown in Figure 6 for variance associated with emission wavelength (A), and excitation wavelength (B). Similar analyses were undertaken for subsets of these data as described above. Specifically, spectra were collected at different times from the same samples. This was undertaken to test for experimental processing artifacts in the data resulting from sample material heterogeneities. Table 2 lists the modeled peak wavelengths of emission and excitation (Figures 6A and B) with the corresponding uncertainties in computed wavelengths of maximum emission and excitation from replicate samples.

Elucidation of effective excitation and emission spectra for each factor can enable assignment of these components to classes of luminescent compounds within the resulting extracts. Good agreement was found with literature values of corresponding peak wavelengths, λ_{max}, for tryptophan residues within polypeptides [25 pp 171], NAD(H) [24 pp 189] or pyridoxic acid (or pridoxic acid 5'-phosphate) [25 pp 189], and coumarins (6,7-dihydroxy and 7-hydroxy-6-methoxy) [25 pp 233]. These assignments are also listed in Table 2. Because of the strongly basic conditions of the PBS extraction solution (i.e. 12.5), it is presumed that acidic moieties would be completely deprotonated. Ionization of these chemical compounds would enhance their respective extraction into the PBS solution.

Armed with these speculative spectral assignments, examination of spectra from the various trash components reveals significant contributions of these factors (Figure 7). Tryptophan, factor 1, contributed to the luminescence signatures of seeds, fiber, and stem materials for upland cotton plants (Figure 7A) while factor 3 (coumarins) was found to contribute significantly to the signatures of bracts and leaf extracts for that species. Factor 2 contributed to most cotton parts other than fiber, seed, bract and leaf. Tables 3 and 4 list the proposed Factor contributions to the spectral signatures of each material investigated for upland and Pima cotton species, respectively. A similar analysis of the samples for Pima cotton indicates Factor 1 dominates fiber spectra and Factor 2 is a major component in stem material extract spectra. All three factors appear to contribute, with varied degrees, to the spectra from the burr of the plant. These assignments may then be applied to the U-PCA cluster analysis of these spectra (Figure 4).

Conclusion

The attempt to separate different botanical components of the

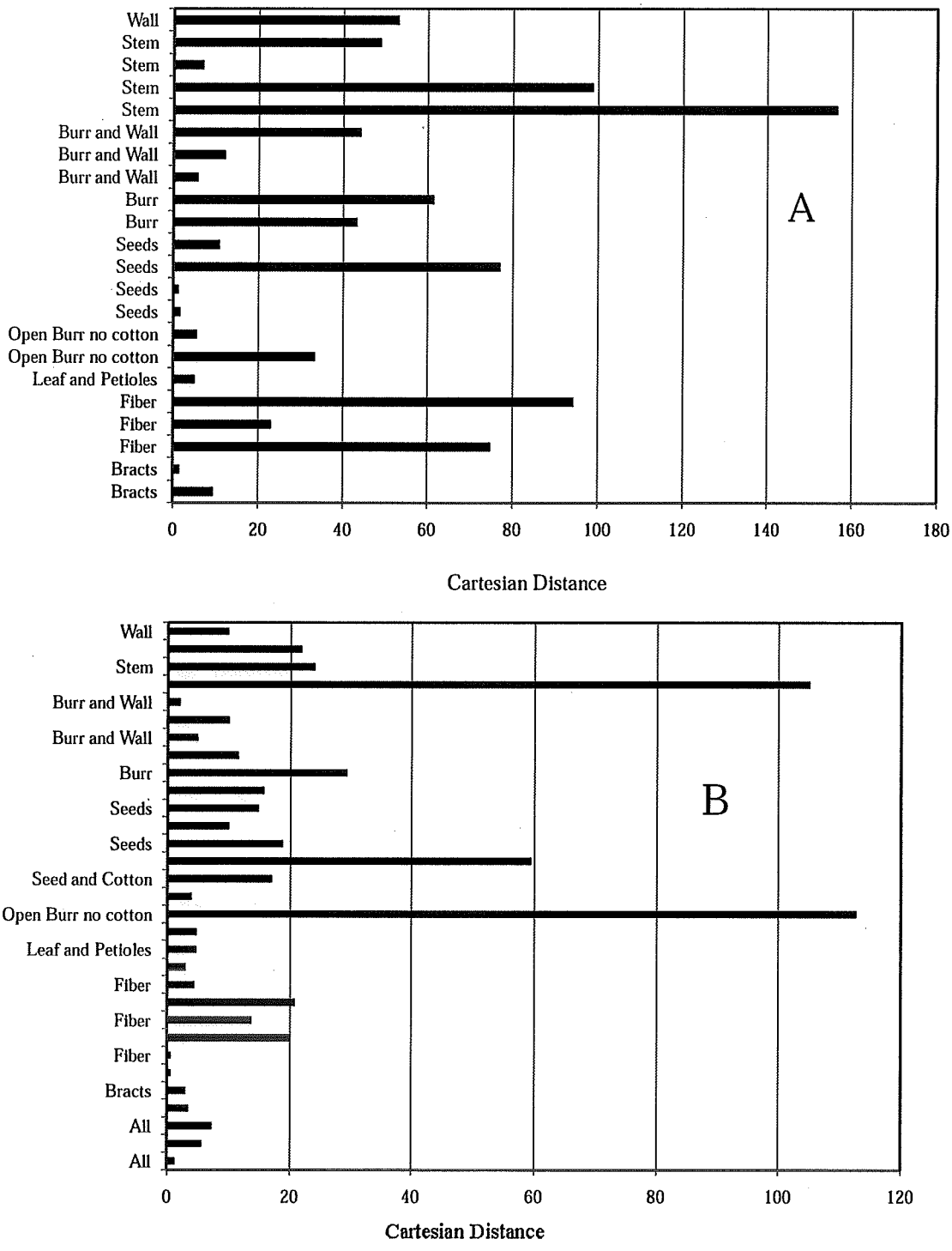

Figure 5: Test of model dependence on sample selection. Mean Cartesian distance for a U-PCA score from one data set applied to the U-PCA model derived from another data set (as per Figure 3). A) Pima cotton plant components and B) upland cotton plant components.

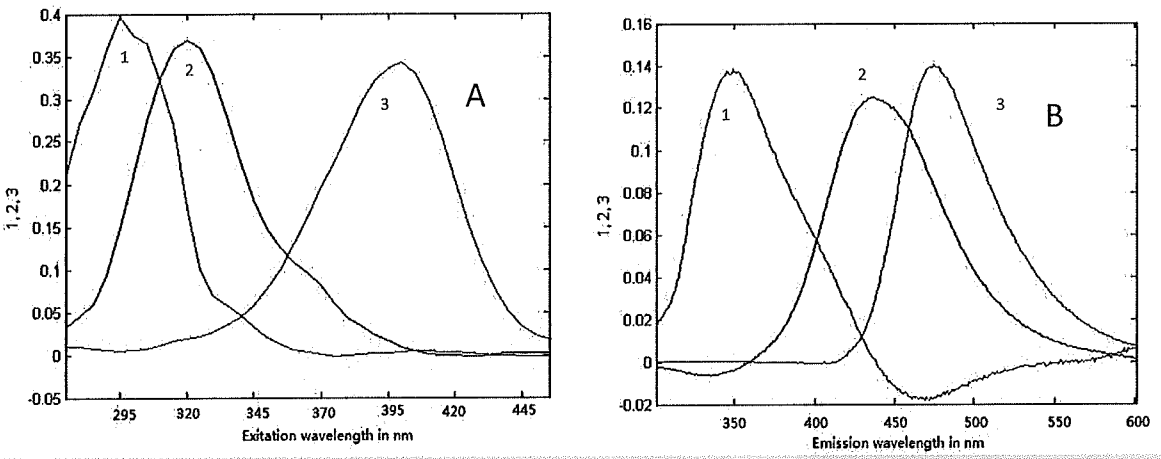

Figure 6: PARAFAC model of emission and excitation luminescence spectra of 12.5 pH PBS extract solutions using three factors. A) Modeled excitation spectrum loadings and B) Modeled emission spectrum loadings for each factor.

Table 2: Excitation and emission wavelengths for individual Factors computed using PARAFAC applied to three-dimensional excitation-emission spectra with corresponding literature values.

Factor	Computed/nm		Literature/nm		Potential Assignments	
	Excitation	Emission	Excitation	Emission	Compound	Reference [25] page number
1	295.±.1	347.7±1.2	287	348	Tryptophan	171
2	320.±.1	435.7±0.9	340 317	450 425	NADH pyridoxic acid and pyridoxic acid-5'-phosphate	189
3	400.±.1	473.3±0.5	390	465	6, 7-Dihydroxy and 7-Hydroxy-6-methoxy coumarins at pH 10	233

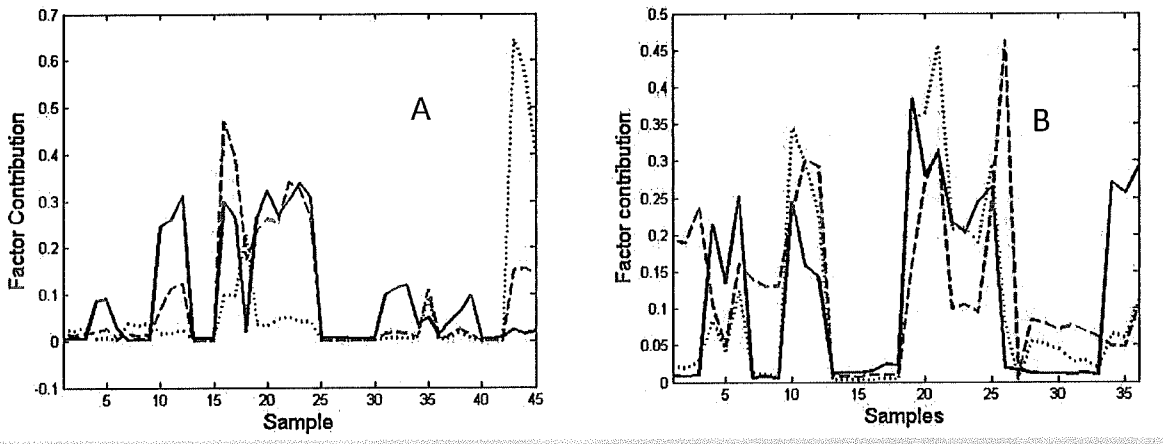

Figure 7: Contribution of factors (Table 2, Figures 6A and B) to each sample for a PARAFAC model of emission and excitation luminescence spectra of PBS extract solutions using three factors applied to materials from A) upland cotton and B) Pima cotton for factors 1(__), 2(- - -), and 3 (...).

cotton plant, representing two different cotton species, through an excitation-emission matrix obtained from an aqueous phosphate buffered saline extract at a pH of 12.5 produced differences as well as similarities. The emission intensity produced as a function of both excitation and emission wavelengths allowed the use of U-PCA procedure to detect differences

The leaf petioles and bracts of upland cotton tends to share many of the same chemical fluorometric properties making their separation

Table 3: Forty-five identification numbers within Figure 7A for relative contribution of each factor (Table 2) for upland cotton materials.

1	Bracts	13	All	24	Outer Burr	36	Seeds
2	Bracts	14	All	25	Fiber	37	Seeds
3	Bracts	15	All	26	Fiber	38	Seeds
4	Fiber	16	Open burr no cotton	27	Fiber	39	Seeds
5	Fiber	17	Open burr no cotton	28	Fiber	40	Burr & Wall
6	Fiber	18	Open burr no cotton	29	Fiber	41	burr & Wall
7	Leaf & Petioles	19	Open Burr	30	Fiber	42	Burr& Wall
8	Leaf & Petioles	20	Open Burr	31	Seed & Cotton	43	Stems
9	Leaf & Petioles	21	Open Burr	32	Seed & Cotton	44	Stems
10	Midwall	22	Outer Burr	33	Seed & Cotton	45	Stems
11	Midwall	23	Outer Burr	34	Seeds		
12	Midwall			35	Seeds		

Table 4: Thirty-six identification numbers for samples within Figure 7B for contributions of each factor (Table 2) for unique samples of Pima cotton materials.

1	Bracts	13	Seeds	25	Stems
2	Bracts	14	Seeds	26	Stems
3	Bracts	15	Seeds	27	Stems
4	Fiber	16	Seeds	28	Burr stems
5	Fiber	17	Seeds	29	Burr stems
6	Fiber	18	Seeds	30	Burr stems
7	Leaf & Petioles	19	Burr	31	Burr
8	Leaf & Petioles	20	Burr	32	Burr
9	Leaf & Petioles	21	Burr	33	Burr
10	Open burr without cotton	22	Burr wall	34	Seeds
11	Open burr without cotton	23	Burr wall	35	Seeds
12	Open burr without cotton	24	Burr wall	36	Seeds

challenging due to clustering and overlapping of these two materials on the score plots. Although the leaf petioles and the bracts of upland cotton plant overlap with each together, the clusters of leaf and bracts were separated from other components of the plant like stem, seeds, fiber, open bolls, open burr, and burr fragments of the cotton plant

The stem material of the both species of cotton (Pima and upland cotton) was easily separated from all other botanical parts of the cotton plant within the 95% confidence interval. This suggests that the model developed appears to be robust to separate residual trash that is likely to be present in the lint during the process of harvesting and ginning when its origin is from the stem of the cotton plant. The model generated also shows potential for the discrimination of other botanical parts of the cotton plant.

These spectral differences are proposed to result from variations in the extraction of tryptophan-containing proteins or polypeptides, NADH or pyridoxic acids, and hydroxyl coumarins.

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