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Quantitative Analysis of Cotton (*Gossypium hirsutum*) Lint Trash by Fluorescence Spectroscopy

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The presence of cotton plant botanical components, or trash, embedded in lint subsequent to harvesting and ginning is an important criterion in the classification of baled cotton by the U.S. Department of Agriculture Agricultural Marketing Service. The trash particles may be reduced in size to the point that specific trash types are not identifiable by image or gravimetric analysis, and it is desirable to quantify different trash types so that processing lines may be optimized for removal of the most problematic trash to enhance processing performance and cotton lint quality. Currently, there are no methods available to adequately quantify cotton lint trash based on botanical origin. The present work attempts to address this issue through the analysis by fluorescence spectroscopy of dimethyl sulfoxide extracts of mixtures of six botanical trash types. The fluorescence data are subsequently subjected to chemometric analysis. The resulting 6 partial least-squares calibration models obtained from 128 mixtures are demonstrated in the case of leaf and hull to be capable of predicting individual trash component concentrations with a high degree of confidence.

KEYWORDS: Cotton (*Gossypium hirsutum*); trash; chemometrics; fluorescence

INTRODUCTION

The presence of cotton plant botanical components (i.e., leaf, stem, hull, shale, seed coat, or bract) in ginned cotton lint is an important criterion for determining cotton quality at the U.S. Department of Agriculture (USDA) Agricultural Marketing Service (AMS) cotton classing offices, which class and grade most bales of cotton produced in the United States for a small fee (*1*). These cotton fiber trash measurements have progressed from being performed by a subjective human classer to the objective High Volume Instrument (HVI) and have a direct bearing upon the perceived value of the bale. Botanical trash is known to negatively affect processing efficiency as well as the quality of finished cotton textile products (*2*, *3*). As a result of this impact upon cotton quality, a number of methods have been developed and utilized to measure trash quantity by various means.

The first instrument for measuring cotton trash was the Shirley Analyzer (SDL International, Stockport, U.K.), which is a destructive gravimetric method that mechanically and pneumatically separates cotton lint and trash. The objective and nondestructive HVI (Uster Technologies Inc., Knoxville, TN) provides a rapid geometric trash measurement at a low cost using a scanning video camera at one set of conditions. This percentage of nonlint surface area is correlated to the classer's leaf grade (1 through 7 and a "below grade"), which is a visual estimate of cotton plant leaf particles in cotton. Recent HVI software developments are able to rapidly quantify cotton trash and provide a particle frequency distribution. The advanced fiber information system (AFIS) (Uster Technologies Inc.) is a destructive method (*4*) that mechanically opens fibers and separates trash for electro-optical measurement, thus producing a trash and dust particle size distribution. None of these methods, however, is able to provide information regarding trash categorization of detected particles according to type. The Cotton Trash Classification (CTC) system (*5*), combined with clustering analysis, has been demonstrated to be capable of categorizing leaf, stem, and seed coat particles on the basis of shape and color, although this method has numerous limitations.

Residual trash embedded in the lint during harvesting and ginning is reduced in size to the point that specific trash types are not identifiable by image or gravimetric analysis. Ideally, one would want to differentiate and classify these ground trash particles so that they are adequately removed to prevent processing problems. This is important because it has been shown that different types of trash are more problematic than others at particular stages of processing. For example, Frey et al. (*6*) found that seed coat fragments were the primary cause of end breaks during yarn production. All spinning systems and their resultant spinning efficiencies depend to some degree upon the percentage of visible foreign matter.

Open-end, or rotor, spinning is particularly sensitive to pepper and dust size trash (*7*). At this stage in processing, residual trash in the sliver has been reduced in size to the point where it can cause a buildup in the rotor groove, thus interfering with the efficiency and quality of rotor yarn. To avoid this rotor groove buildup and thereby enhance processing efficiency, it is desirable to know whether specific types of trash are present at a higher

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concentration than others. With this knowledge, mitigation of rotor buildup may be possible through optimization of ginning and the sequence of textile opening and cleaning equipment prior to spinning aimed at the reduction of specific botanical plant parts.

Trash particles can be difficult to locate, measure, and describe because trash can arise from many components and can be irregularly sized, erratically positioned, partly covered by cotton fibers, or light colored in nature. Botanical trash particles originate from the cotton plant with various parts of the leaf, stem, bark, seed, and hull. Each of these plant tissues is unique, exhibiting complex mechanical structures and chemical compositions. Wall structure and composition differ between tissues from thin to thick with diverse density levels. Goynes and Berni (*8*) wanted to differentiate plant parts for dust studies and attempted to determine evident variations in elemental content among plant parts from five different growing regions of the United States during three growing seasons. They also found that a calcium/potassium relationship could be utilized for leaf, bract, stem, pericarp, and seed coat plant part differentiation and classification.

Identification of specific trash components exhibiting small particle sizes must rely on chemical/spectroscopic characteristics unique to each trash component. Previous work (*9*) utilizing FTIR spectroscopy has been conducted with the goal of identifying specific trash types on the basis of quantitative differences in chemical functional groups such as −OH, −CH, and −CO. An inherent difficulty of this approach arises in that all of the botanical trash components display very similar FTIR spectra due to the fact that each of the botanical trash types is composed of similar quantities of abundant components including cellulose, wax, and pectin. A further complication in attempting to compare these types using FTIR arises due to changes in particle size and water content, which occur as a result of cotton processing. Both of these phenomena have been demonstrated to affect the resultant FTIR spectra (*10*), consequently making identification of trash types reliant upon knowledge of their complete processing history.

Many natural compounds derived from plants, such as chlorophyll, exhibit fluorescence, and it may be expected that the concentration of fluorophores in a plant will be variable between tissue types due to the specific physiological function of the molecules. As an example, chlorophyll is present in higher amounts in leaf tissue than in other tissues. Using fluorescence, Himmelsbach et al. (*11*) have suggested that the outer pigment layer of the cotton seed coat contains anthocyanin or proanthocyanidin compounds. Fluorescence spectroscopy has been utilized previously in the analysis of a wide variety of agricultural commodities, including the differentiation of red and white wheats (*12*) and harvest time information in apples (*13*). In addition to exhibiting a high degree of sensitivity and specificity, fluorescence spectroscopy also allows for the acquisition of multivariate data, which provides a means by which correlations between a large number of variables having incompletely understood interrelationships may be used to develop a predictive model.

The present work attempts to utilize differences in chemical compositions among trash types that may be unobservable by FTIR due to its lack of sensitivity to relatively minor constituents. One technique that does display a high sensitivity to very small amounts of certain analytes is fluorescence spectroscopy. This work describes a multivariate calibration approach based on partial least-squares (PLS) regression to predict percent composition in samples composed of blends of leaf, stem, hull, bract, shale, and seed coat trash types using fluorescence spectra of dimethyl sulfoxide (DMSO) extracts.

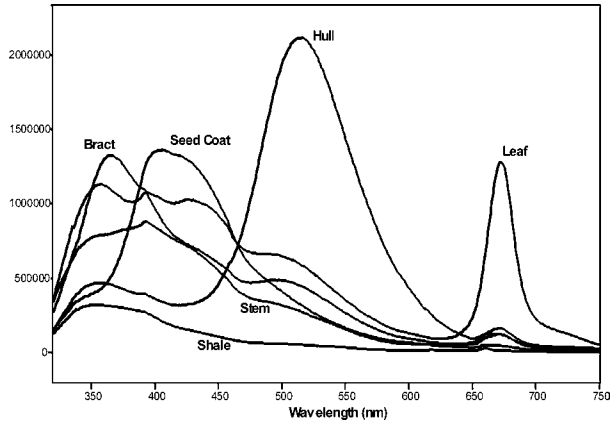

Figure 1. Fluorescence spectra of DMSO extracts of the six trash types used in this study. $\lambda_{ex} = 300$ nm.

Table 1. Number of Factors Used for Each Trash Component in the PLS1 Calibration Model, with *F* Ratio, R^2, SECV, and SEP Values for Each Factor

constituent	factors	*F* ratio	R^2	SECV (%)	SEP (%)
leaf	16	1.10	0.93	6.4	5.8
seed coat	8	1.06	0.58	16.0	NA
hull	12	1.12	0.94	6.2	5.4
shale	10	1.00	0.43	19.7	NA
bract	14	1.09	0.73	13.8	NA
stem	15	1.01	0.79	11.9	NA

MATERIALS AND METHODS

Cotton Trash Samples. Botanical cotton trash samples used for the development of the calibration set were collected from three varieties of cotton: Delta Pine Land-33B (DP33B), grown in Texas during the 1999 crop year; Stoneville-6611 (SV6611), grown in South Carolina during the 2006 crop year; and Delta Pine Land-449 (DP449), grown in South Carolina during the 2006 crop year. Leaf, stem, hull, shale, and bract were separated from the whole plant by hand. Seeds were collected by ginning the bolls on a microgin, following which the seeds were delinted using sulfuric acid (*10*) and the seed coats were subsequently manually removed from the seed meats. The sulfuric acid treatment enhances the efficiency with which seed coats may be removed from the seed meat, and DMSO extracts of seed coats not subjected to sulfuric acid treatment show no difference in fluorescence from the treated seed coats. Each of the isolated trash components was then milled to a 1 mm mesh size. One hundred and twenty-eight mixtures of the milled components, representing a wide range of possible relative concentrations, were prepared by adding various concentrations of each component to a total weight of 0.100 g. Thirty additional samples including each of the varieties used to prepare the calibration set were prepared and used as validation samples.

Extraction. Samples of the isolated plant trash components as well as the series of mixtures were weighed to 0.100 g and extracted with 20 mL of DMSO (Aldrich Chemical Co., Milwaukee, WI) for 2 h at room temperature, following which the extracts were filtered (0.45 μm) from the residue and immediately analyzed by fluorescence spectroscopy.

Fluorescence Spectroscopy. The DMSO extracts were diluted 4:1 with DMSO prior to collection of fluorescence spectra to avoid attenuation of the signal due to self-absorption. Fluorescence spectra (320−750 nm) were acquired using a Fluorolog3-121 (Jobin-Yvon Spex, Edison, NJ) with 300 nm excitation. The exit slit of the excitation monochromator and the entrance slit of the emission monochromator were set at 2 nm bandwidth, and spectra were acquired using a 0.5 s integration time.

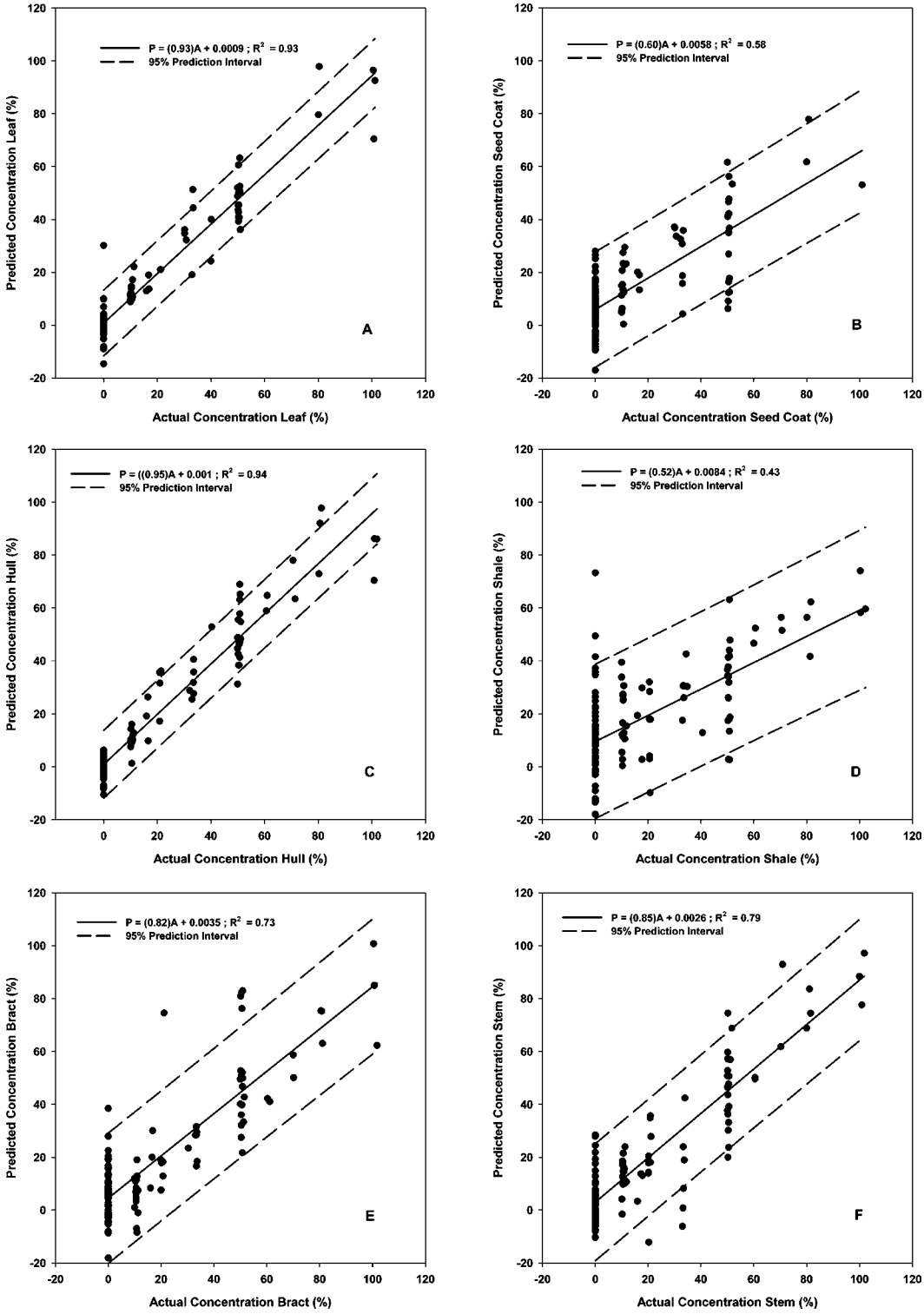

Figure 2. Scatter plots of predicted (*Y*) versus reference (*X*) values for the amounts of leaf (**A**), seed coat (**B**), hull (**C**), shale (**D**), bract (**E**), and stem (**F**) in a mixture of all six trash types obtained by cross-validation.

Chemometrics. A multivariate calibration file was developed using the PLS1 program of the PLSPlus/IQ chemometric module for GRAMS/AI (Galactic Industries, Inc., Salem, NH), and all statistical analyses were performed with the same software. Two spectral regions, 335−592 and 611−750 nm, were used in the calibration model, and all spectra were mean centered. Evaluation of model performance was tested by cross-validation, and no outliers were removed.

RESULTS AND DISCUSSION

Individual fluorescence spectra of the six trash components analyzed are presented in **Figure 1**. Although each of the components exhibits a characteristic fluorescence spectrum, there is no region across the entire range of the spectrum that is unique to any one component, thus necessitating development of a multivariate calibration model. Attempts at extracting the trash samples using solvents other than DMSO, including ethanol, acetone, chloroform, and water, resulted in representative spectra that did not display the degree of distinctiveness exhibited by DMSO extractions. The calibration model presented here is based on PLS. A training set of 128 fluorescence spectra representing a range of concentration ratios, cotton plant

varieties, growing location, and ages was used to build a calibration model. The resulting model was optimized by cross-validation: each of the 128 spectra was sequentially removed from the training set, and the concentration of the six trash components was predicted from the remaining 127 spectral files. **Table 1** shows the *F* ratio, standard error of cross-validation (SECV), and coefficient of determination (R^2) versus the number of factors for each of the six trash components. The resulting optimized calibration model uses 16 factors for leaf, 8 factors for seed coat, 7 factors for hull, 10 factors for shale, 14 factors for bract, and 15 factors for stem. Plots of predicted concentration (from cross-validation using the number of factors indicated above) versus actual concentration are shown in **Figure 2** for each of the trash components.

Both leaf and hull (panels **A** and **C**, respectively, of **Figure 2**) exhibit linear relationships between actual and predicted values that describe the data well, as evidenced by R^2 values of 0.93 and 0.94, respectively. The high degree of correlation found with these two trash constituents is due to their characteristic fluorescence spectra. Hull exhibits a band centered at ~510 nm that is very intense relative to the other five constituents, whereas leaf displays an intensity centered at ~670 nm that is very strong relative to the remaining constituents. The remaining four constituents, seed coat, shale, bractm and stem, are more difficult to characterize on the basis of comparison of their fluorescence spectra, and the calibration models produced for these four components (panels **B**, **D**, **E**, and **F**, respectively, of **Figure 2**) do not appear to exhibit correlations between actual and predicted values sufficiently strong to justify using the models for predictive purposes on unknown samples. A validation set of samples that included 30 mixtures of the 6 trash components for all three cotton varieties were extracted in DMSO and their fluorescence spectra acquired. These fluorescence spectra were subsequently analyzed using the developed calibration models for leaf and hull. The resulting standard error of prediction (SEP) was comparable the SECV obtained in the calibration model (**Table 1**). This suggests that the developed calibration models for both leaf and hull are capable of reliably predicting trash concentrations of different cotton varieties from different growing regions with a high degree of confidence. Models for the remaining four trash components may potentially be improved by expansion of the calibration sets, and this is the subject of ongoing work.

Botanical trash particles are difficult to differentiate because trash can arise from many components and become reduced in size throughout processing. These trash particles originate from the cotton plant from various parts of the leaf, stem, bark, seed, and hull and thus contain different chemical compositions and complex mechanical structures. The developed calibration model presented here eliminates many of the problems associated with the quantification of different trash types. The differentiation of leaf, stem, hull, shale, bract, and seed coat plant parts presented here is based upon the characteristic fluorescence spectra of DMSO extracts of the individual trash types. Although none of the trash components uniquely exhibits significant intensity in any region of the spectrum without interfering fluorescence from the other components, there is sufficient difference between the fluorescence spectra to allow for analysis by multivariate methods, in this case PLS1. The results presented suggest that leaf and hull, when subjected to analysis by the developed PLS1 models, can be quantified with a high degree of confidence. Future work will entail expansion of the calibration model to include trash components from new varieties and growing regions to further optimize the precision with which the model predicts trash type quantities. Subsequent studies will evaluate the usefulness of the developed models for leaf and hull in identifying their relative concentrations at the cotton gin and subsequent textile-processing points to identify whether these trash types are problematic.

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