Invisible Coatings for Wheat Kernels

M. S. Ram,[1,2] Floyd E. Dowell,[1] and Larry Seitz[3]

Cereal Chem. 79(6):857–860

ABSTRACT

It is occasionally necessary to tag wheat kernels without altering their appearance. Coatings have potential applications to tag wheat of a particular color or protein class, diseased wheat such as Karnal bunt, or genetically modified wheat. This methodology will aid in development of calibrations for sorting instruments. Procedures were developed to coat wheat kernels with invisible ultraviolet (UV) fluorescent and near-infrared (NIR) absorbing noncarcinogenic dyes. Wheat coated with UV-fluorescent compounds were identified under black light. The NIR-absorbing coating required lower concentrations of dye than the UV dyes and wheat coated with NIR-absorbing dye were identified from their NIR spectrum.

It is occasionally necessary or beneficial to tag wheat kernels without altering their appearance. For example, some cultivars of red and white wheat have similar color and are difficult to identify when mixed. If one class was tagged with a coating, then mixtures could be readily identified with the use of special lighting or sensors. Similarly, it may be desirable to tag wheat of a particular protein class to evaluate the efficiency of sorting instruments. In fact, the work described here was developed in response to such a request (Ram et al 2001).

Also, some industry segments may be interested in tagging genetically modified wheat, wheat identified as having some type of damage, treated wheat, or wheat that needs to have its identity preserved. Invisible coatings would provide a means to tag wheat for subsequent analysis by use of sensors that are able to detect such coatings.

This methodology will also aid in development of calibrations for near-infrared (NIR) and visible wavelength sorting instruments. Wheat classes, such as red versus white, high protein versus low protein, kernels infected with Karnal bunt versus uninfected, or genetically modified cultivars versus nongenetically modified cultivars, may possibly be sorted based on the difference in their spectra, and the sorting efficiency determined by the fluorescence of the coating applied to the kernels with the attribute to be removed. Wheat coated using procedures described here probably cannot be used for food but may be used for seeds. Most recently, the invisible coating was used to tag kernels infected with Karnal bunt during calibration of high-speed sorting instruments (Dowell et al 2002).

Coatings have several advantages over chemical derivatization. Coatings are reversible and could be stripped mechanically or with solvents. Very strongly adherent coatings are possible with the use of binders such as clear-coat lacquer or matte finish paints. Coatings do not change physical or chemical properties of the surfaces coated. Conditions required for substantial surface chemical derivatization may be harsh and difficult to realize. Derivatization often affects physical properties of the surface. In wheat, the pericarp tended to buckle, shrink, and fall off, leaving the kernels discolored upon derivatization. Coatings, on the other hand, can be applied rapidly. The objective of this research was to investigate invisible coatings and how to distinguish coated from uncoated kernels by visible and spectrometric methods.

[1] Engineering Research Unit, USDA-ARS, Grain Marketing and Production Research Center, 1515 College Ave, Manhattan, KS 66502. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

[2] Corresponding author. Phone: 785-776-2730. Fax: 785-776-2792. E-mail: ramms@gmprc.ksu.edu.

[3] Grain Quality and Structure Research Unit, USDA-ARS, Grain Marketing and Production Research Center, 1515 College Ave, Manhattan, KS 66502.

Publication no. C-2002-1007-07R.

MATERIALS AND METHODS

Ultraviolet (UV) fluorescent compounds chrysene, benzo[a]pyrene, pyrene, naphthacene, pyrene-1-sulfonic acid sodium salt, HPTS (1-hydroxypyrene-3,6,8-trisulfonic acid trisodium salt), coumarin I (7-diethylamino-4-methylcoumarin), and a near-infrared (NIR) absorbing chemical NCSi (naphthalocyanine(bis-trihexylsiloxy) silicon) (Fig. 1) were obtained from Aldrich Chemical (Milwaukee, WI). Invisible UV inks (UV50 in types A, B, and D), UV lacquer (UV11), and invisible UV water-base paint (UV21) were obtained from Theatre Effects (Hagerstown, MD). Spray paint cans of Premium Decor clear lacquer and Krylon matte finish were obtained from local hardware stores.

Coatings with chrysene, benzo[a]pyrene, pyrene, naphthacene, and coumarin I were made by dissolving 5 mg of each in one or two drops of acetone, mixing with 3 g of wheat, stirring with a spatula and letting the samples dry on petri dishes in a fume hood overnight (amounts of materials described here did not need to be exact because the coatings were generally used for only distinguishing coated from uncoated wheat kernels). Coated samples were separated from the dust with tweezers. Water or DMSO (dimethylsulfoxide) was used as the solvent instead of acetone for pyrenesulfonate and HPTS coating. CH_2Cl_2 was used as the solvent for NCSi coating. Only 3 mg of NCSi was used to coat 30 g of wheat.

Adherent coatings were made by mixing 0.5 mL of lacquer paint with 30 g of wheat and then adding 50 mg of coumarin I in 0.5 mL acetone. To eliminate the slight gloss in the coatings due to the lacquer, the kernels were sprayed with 0.5 mL of matte finish paint and stirred with a spatula and dried overnight. Applying gentle pressure broke up clumps, and coated kernels were separated from dust with tweezers.

Coatings with UV lacquer (UV11) were made by mixing 10 g of wheat with 1 mL of lacquer. The lacquer tended to settle at the bottom and had to be stirred before use. Coatings with invisible inks (UV50) were obtained by coating the kernels with paint brushes if only a few kernels were to be coated. Otherwise, 30 mL of UV ink was mixed with 30 g of wheat, stirred for ≈2 hr, drained of excess liquid. The coated kernels were left to dry in a fume hood overnight, and separated from the dust with tweezers. Coatings with UV paint (UV21) were made by mixing wheat with the required amount of paint. Coated kernels were dried and separated.

Fluorescence viewing. A Spectroline CX-20 UV cabinet and a Spectroline battery-operated portable UV lamp from Aldrich were used for viewing fluorescence of coated samples. Long wavelength UV light (360–400 nm), referred to as black light, was used for viewing the fluorescent coatings. Because of its low intensity, the portable light had to be held very close to the sample.

Fluorescence spectra. Fluorescence emission spectra were obtained using a FluoroMax-2 spectrofluorometer (Jobin Yvon SPEX, Edison, NJ), using 1-nm resolution and 0.1-sec integration time. The instrument had two monochromators for excitation and emission. Instrument control and data acquisition were handled by a computer. Spectra were saved in Grams/32 software program (Thermo

Galactic, Salem, NH). Fluorescence spectra of wheat kernels were obtained using 10-mm path length quartz spectrophotometric cuvettes. The excitation maximum for coumarin I was ≈390 nm. However, even with 350-nm excitation, fairly good signal was obtained enabling fluorescence data collection at 370–900 nm.

NIR spectra. A diode-array NIR spectrometer (Perten Instruments, Springfield, IL) was used to collect spectra from single wheat kernels. Spectra were obtained for uncoated kernels, and kernels coated with NCSi, UV50, and coumarin I. Single kernels of wheat samples were placed in a black V-shaped trough (12 mm long × 10 mm wide × 5 mm deep). Trough sides of the bucket were at 45° angles from vertical. Baseline spectra were taken with the empty black bucket. The black bucket gave a flat baseline in the entire spectral range. Kernels were placed with the crease down and germ end toward the observer in the black viewing bucket with tweezers to minimize errors from varying orientations. Touching the kernels was avoided. White light illuminated a single kernel through an 8 × 60 mm (diameter by length) fiber optic bundle at a 45° angle to the sample bucket. A 2 × 90 mm fiber optic bundle transmitted the kernel reflectance to the diode array. Fifteen spectra were collected from each kernel and averaged to reduce noise. The spectra were stored on a hard disk for subsequent analysis. For data analysis, log (1/*R*) (*R* = sample reflectance/background) in the 400–500 nm range was discarded due to excessive noise.

PLS analysis. NIR data collected from uncoated and coated kernels were analyzed using partial least squares (PLS) analysis (Martens and Naes 1989). All data were mean-centered, which meant calculating the average of all spectra in the training set and then subtracting the result from each spectrum. Spectral data were analyzed by the Grams/32 software PLS model using the absorbance data at 500–1,700 nm at 5-nm intervals (*x*-data), and appropriate constituent values (*y*-data) were analyzed using a full cross-validation scheme. Two sets of PLS analyses were made. In one set, spectra of uncoated wheat were analyzed against UV50-coated wheat, assigning a constituent value of 1 for uncoated and 2 for UV-coated wheat spectra. In another set, spectra of uncoated wheat were analyzed against NCSi-coated wheat, assigning a constituent value of 1 for uncoated and 2 for the NCSi-coated wheat spectra. The number of factors reported was obtained from the predicted residual error sum of squares (PRESS) versus the number of PLS factors plot corresponding to the lowest PRESS value obtainable with the least number of factors. This number of factors, which was close to the software recommended value, was evaluated as acceptable from actual versus predicted and β-coefficient versus wavelength plots.

RESULTS AND DISCUSSION

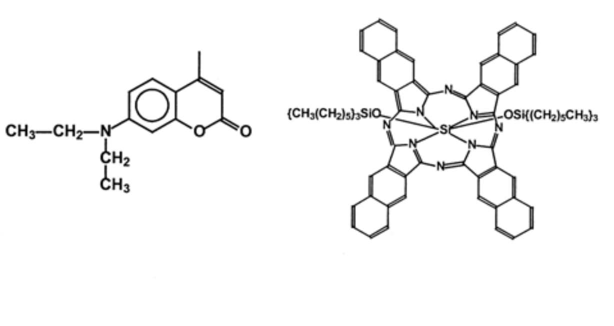
Samples coated with the polyaromatic hydrocarbons mentioned above showed good fluorescence but these fluorophores are carcinogenic. Wheat kernels coated with pyrenesulfonates in water or DMSO lost their fluorescence once the solvents evaporated. The fluorescence of HPTS was blue in DMSO and green in water. Samples coated with UV lacquer (UV11) had excellent bluish-white fluorescence but the samples had a glossy appearance. Wheat coated with Type A invisible ink (UV50) for porous materials tended to darken the kernels. UV coatings with the UV paint (UV21) had a chalky white appearance.

UV coatings with invisible ink (UV50), Type B or D, and UV coatings made with coumarin I were nontoxic, adherent, and invisible. Type B is for nonporous materials, and Type D is indelible ink for body stamping. Invisible inks contain glycerin, water, and dyes certified for drugs and cosmetics and can be washed off with soap and water. UV-fluorescent coatings were stable for several weeks in ambient light. NCSi (Fig. 1) is also nontoxic and is used in medical applications (Brasseur et al 1994). NCSi has also been used in security applications for invisible bar code printing (Yousaf and Lazzouni 1995). The NIR spectra of wheat coated with NCSi faded completely within a week. Other NIR dyes (Stoyanov 1995) may have better stability.

Under ambient light UV-coated, NCSi-coated, and uncoated wheat appeared identical; but in the UV viewing cabinet, the UV-coated wheat had a bright blue fluorescence. NCSi-coated wheat could easily be distinguished by taking an NIR spectrum of the wheat sample.

Spectra of Coated and Uncoated Wheat

The fluorescence emission spectra of coumarin I coated and uncoated wheat for excitation at 350 nm are given in Fig. 2A. The excitation maximum was ≈395 nm. The emission maximum for



Coumarin I **NCSi (Naphthalocyanine (bis-trihexylsiloxy)silicon)**

Fig. 1. Structures of UV-fluorescent and NIR-absorbing compounds used in coatings for wheat.

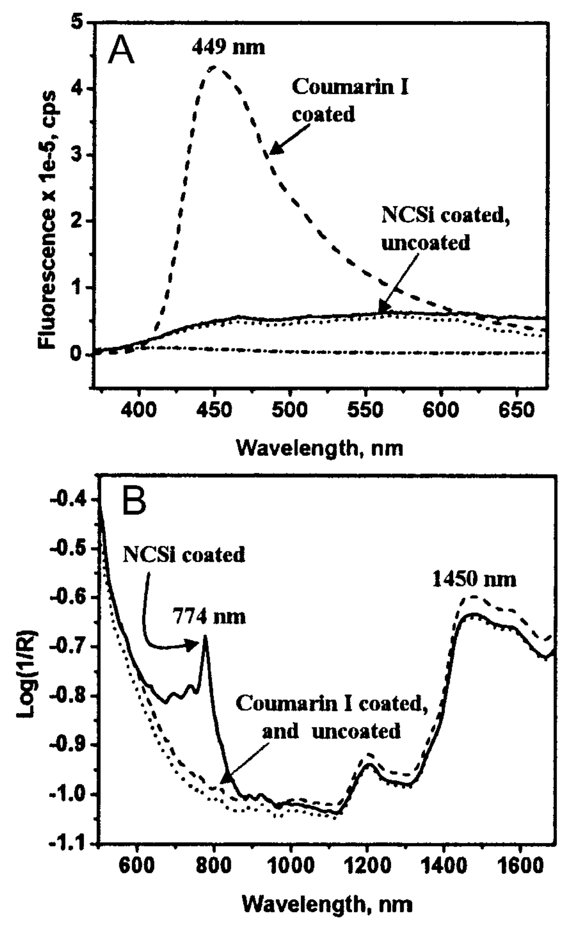


Fig. 2. A, Fluorescence emission spectra of NCSi-coated wheat (—), coumarin I-coated wheat (- - - -), uncoated wheat (• • • •), and empty cuvette (–•–•–)at 350 nm excitation. **B,** Visible-NIR spectra of NCSi-coated wheat (—), UV-coated wheat (- - - -), and uncoated wheat (• • • •) .

the coumarin I-coated wheat kernels was 449 nm, corresponding to the blue fluorescence. Uncoated and NCSi-coated wheat exhibited weak fluorescence emission spectra, apparently due to the natural fluorescence of wheat from compounds like ferulic acid. The empty quartz cuvette did not have this weak emission spectrum.

UV-fluorescent coatings had very little influence on the visible NIR spectra of wheat. However, coated wheat kernels could be identified from their NIR spectra by multivariate analysis such as PLS. NCSi-coated wheat had distinct spikes at 734 and 774 nm (Fig. 2B). These peaks were from the NIR coating compound at only ≈70 ppm. Water, protein, and carbohydrates present in the wheat at more than 1,000× this level made less contribution to the total absorbance.

PLS Analysis

Coated wheat was easily discriminated from uncoated wheat in PLS analysis of NIR spectra (Table I), as judged by various plots and parameters. Convergence was achieved with only four factors for the NIR-coated wheat, 12 factors for UV50-coated wheat, and six factors for coumarin I-coated wheat (data not shown). It was interesting to note that classification of UV50 or coumarin I coated wheat was also good, even though there was not a huge spectral difference between coated and uncoated wheat (Fig. 2B). This was perhaps because the invisible, UV-coating compounds have weak NIR spectra, which can be distinguished in PLS analysis. A plot of β-coefficients versus wavelength was noisy, and the peak values are indicated in Table I. However, classification of UV50-coated wheat or coumarin I-coated wheat versus uncoated wheat was nearly 100%. That the classification of NCSi-coated wheat by PLS was also excellent was not a surprise because the NIR spectrum of the NCSi-coated wheat was visibly different from that of uncoated wheat (Fig. 2B). Also, a plot of β-coefficients versus wavelength, which was not noisy in this case, showed a similarity to the NIR spectrum of the coating itself (Fig. 3). The only other peak in the β-coefficient plot was that of moisture at 1,450 nm. This demonstrated the significance of a plot of β-coefficients versus wave length in general in that it was expected to resemble the NIR spectrum of the analyte in chemometric analysis.

NCSi vs. UV50 Coating

Both of the coatings were invisible. However, UV50-coated wheat can be visualized by fluorescence under black light, which is avail able in hand-held sizes. Autofluorescence was a problem, and high concentrations of the coatings were necessary because most materials had traces of autofluorescence. UV50-coating had very little influence on the visible-NIR spectra of wheat. This can be an advantage when calibrating for Karnal bunt sorting (Dowell et al 2002). UV50 or coumarin I coatings were stable for at least two to three weeks under ambient conditions. NCSi-coated wheat kernels require NIR sensor detection, but the coating can be applied in lower concentrations (3 mg/30 g of wheat) than the UV compounds (50 mg/30 g of wheat). There were obvious NIR spectral differences between NCSi-coated and uncoated wheat. There was very little autofluorescence from other substrates in the NIR. NCSi coatings faded within a week.

The cost of materials to coat 1 kg of wheat is under $100. UV lights cost $25–$1,200, but this is not a recurring expense. Detecting NCSi coatings is more expensive. NIR detectors may cost $5,000–$20,000. The process of coating is extremely simple and takes ≈4 hr of work over two days. The concentrations of the coatings are in ppm levels. UV coatings would not add enough nitrogen to affect protein analysis. These coatings may also be useful for tagging insecticide-treated wheat.

CONCLUSIONS

Procedures were developed to coat wheat kernels with UV-fluorescent, and NIR-absorbing (NCSi) noncarcinogenic dyes. All coatings were invisible. Wheat with UV-fluorescent coatings were identified under black light. UV-fluorescent coatings did not have much influence on the visible-NIR spectra of wheat. NCSi-coated wheat was identified from its NIR spectrum. Wheat with NCSi coatings and UV-fluorescent coatings were classified against uncoated wheat by PLS analysis of their NIR spectra. A plot of β-coefficient versus wavelength for PLS analysis of NCSi-coated versus uncoated wheat was similar to the spectrum of the coating itself, demonstrating that such plots, in general, provide the NIR spectrum of the analyte. Coatings have potential applications to tag wheat of a particular color or protein class, wheat with diseases such as Karnal bunt, or genetically modified wheat. The sorting efficiency of similar looking kernels by sorting equipment can be evaluated by the use of coatings. This methodology will aid in development of calibration for sorting equipment.

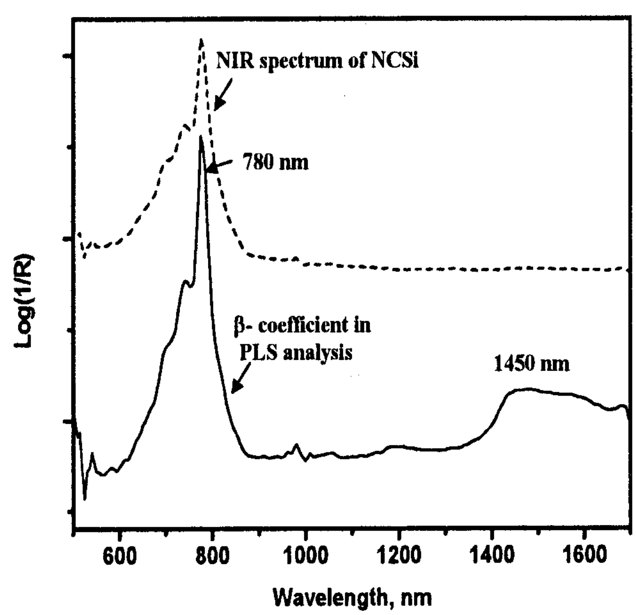


Fig. 3. Visible-NIR spectrum of NCSi and a plot of β-coefficient (for four factors) in the regression analysis of visible-NIR spectra of NCSi-coated kernels vs. uncoated kernels.

TABLE I
Summary of Partial Least Squares (PLS) Analysis of NIR Spectral Data

PLS Parameters[a]	NCSi-Coated vs. Uncoated Wheat	UV50-Coated vs. Uncoated Wheat
Number of spectra	120	219
r^2	0.95	0.75
SECV	0.12	0.251
Factors	4	12
β-Coefficient peaks (nm)	745, 780, 1,065, 1,200, 1,450	550, 590, 645, 705, 770, 850, 940, 975, 1,185, 1,330, 1,465, 1,555, 1,660
% Correctly classified	100	99

[a] Respectively: number of samples used in the analysis; correlation coefficient; standard error in cross-validation; number of factors used; peak values of regression coefficient vs. wavelength.

LITERATURE CITED

Brasseur, N., Nguyen, T. L., Langlois, R., Ouellet, R., Marengo, S., Houde, D., and Van Lier, J. E. 1994. Synthesis and photodynamic activities of silicon 2,3-naphthalocyanine derivatives. J. Med. Chem. 37:415-420.

Dowell, F. E., Boratynski, T. N., Ykema, R., Dowdy, A. K., and Staten, R. T. 2002. Use of optical sorting to detect wheat kernels infected with *Tilletia indica*. Plant Dis. 86:1011-1013

Martens, H., and Naes, T., 1989. Multivariate Calibrations. John Wiley and Sons: Guildford, England.

Ram, M. S., Seitz, L., and Dowell, F. E. 2001. Invisible coatings for wheat kernels. 2nd Int. Wheat Quality Conf. Grain Industry Alliance: Manhattan, KS.

Stoyanov, S. 2001. Probes: Dyes fluorescing in the NIR region. Pages 35-93 in: Near-Infrared Applications in Biotechnology. R. Raghavachari, ed. Marcel Dekker: New York.

Yousaf, M., and Lazzouni, M. 1995. Formulation of an invisible infrared printing ink. Dyes Pigments 27:297-303.

[Received April 12, 2002. Accepted August 12, 2002.]