Use of Fluorometry for the Determination of Uric Acid in Grain. Elimination of Interfering Fluorescence[1]

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ABSTRACT

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The possible use of fluorometry for determining the amount of uric acid in grain, to measure insect contamination or monitor infestation was investigated. In 0.20*M* sodium phosphate buffer, pH 12.3, the excitation maximum for the fluorescence of uric acid was 342 nm, and the emission peak was at 408 nm. In alkaline solution, the fluorescence at 408 nm was independent of pH over the range of 11-12.5. Fluorescence at 408 nm was a linear function of uric acid concentration to several hundred parts per million with a lower limit of detection in the range of tenths of a part per million, a sensitivity sufficient for the analysis of uric acid in commercial grain samples. Direct fluorometric analysis of uric acid was not successful as a result of the presence in grain extracts of an interfering fluorescence with an excitation maximum at 290 nm and an emission peak at 354 nm. The interfering fluorescence was shown to be due to the extraction, at the pH of ~9 used to extract the uric acid, of tryptophan-containing proteins and peptides. Most of the interfering fluorescence was from proteins with molecular weights above 5,000, but a significant portion was due to compounds with molecular weights below 500. Quantitative removal of the interfering fluorescence was achieved by a cation-exchange procedure using Supelclean LC-SCX solid-phase extraction tubes, which contained 3-propylsulfonyl groups chemically bonded to 40-μm silica particles with 60-Å pores.

Insect infestation of stored grain has long been a major problem. It affects storability of the grain, can produce serious losses during storage, and results in infestation and contamination of products made from the grain. Infestation usually is monitored by visual inspection, and contamination is determined by insect fragment count. Both methods are somewhat subjective, and it would be desirable to at least supplement them by more objective procedures. Numerous chemical methods have been considered.

The infrared method proposed for monitoring insect infestation by measuring carbon dioxide produced by the insects (Bruce and Street 1974, Street and Bruce 1976) is complicated by the difficulty in determining the background level of carbon dioxide from respiration of the grain. A procedure is available for determining chitin (Hackman and Goldberg 1981), a major structural component of insect cuticle, but chitin cannot be used as an index of insect infestation or contamination because of the high concentrations found in fungi that infest stored grain (Donald and Mirocha 1977). A colorimetric method for determining 3,4-dihydroxyphenylacetic acid, proposed as a measure of insect contamination of grain (Potter and Shellenberger 1952), has not been used, apparently because of its long analysis time.

One objective procedure that can be used to measure insect contamination or monitor infestation is the determination of uric acid, which is the principal end product of nitrogen metabolism of almost all terrestrial insects and accounts for more than 80% of the nitrogen of their excreta. An early colorimetric procedure for measuring insect uric acid (Subrahmanyan et al 1955, Venkat Rao et al 1957b), based on the arsenophosphotungstic acid reagent of Benedict and Franke (1922), gave a reasonably good correlation between uric acid concentration and the number of insect exit holes in the kernels of the sample (Venkat Rao et al 1957a). Specificity later was improved by incorporation of the enzyme uricase into the procedure (Venkat Rao et al 1959) and by the use of paper chromatography (Venkat Rao et al 1960). Using a slightly modified version of the uricase procedure, Pixton (1965) concluded that it lacked the sensitivity to monitor all but the highest levels of infestation. Despite this limitation, however, the method is still in use in areas where tropical conditions favor high levels of infestation (Joshi et al 1985).

Farn and Smith (1963) reported an enzymatic-ultraviolet method for determining uric acid in flour, which was simpler than the enzymatic-colorimetric procedure; and an improved, more sensitive version (Sen and Smith 1966, Sen 1967), in which hydrochloric acid was used to destroy uricase activity in the sample, was applicable to a variety of cereal products. The lower limit of measurement was 32 $\mu g\ g^{-1}$ of sample, a limit too high to detect levels commonly encountered in commerce. A modification, which included a concentration step (Sen 1968, Sen and Vazquez 1969), extended this lower limit to 0.5-0.6 $\mu g\ g^{-1}$, but the procedure has not been used widely because of its long analysis time.

For the rapid analysis of large numbers of samples, semiautomated versions of a colorimetric procedure based on phosphotungstic acid have been developed. Laessig et al (1972) described a procedure for determining uric acid in flour and cereal grains, which eliminated turbidity problems by dialysis but had a sensitivity of only 40 μg^{-1}. A similar procedure for determining insect infestation of food (Roy and Kvenberg 1981) using immobilized uricase afforded both better specificity and higher sensitivity, and sensitivity was improved further in a modification for the analysis of flour extracts (Galacci 1983).

Recent innovations with improved sensitivity include the use of an oxygen-sensitive electrode for measuring uric acid in spices by its uricase-catalyzed degradation to allantoin (Brown et al 1982) and analysis by high-performance liquid chromatography both with electrochemical (Kissinger et al 1974) and ultraviolet (Cox et al 1976) detection. The potential of high-performance liquid chromatography for determining uric acid in grain has been demonstrated by Pachla and Kissinger (1977) and Wehling and Wetzel (1983). When wheat kernels internally infested with insects at a given stage of development were mixed with sound kernels, there was a linear relationship between uric acid concentration as measured by high-performance liquid chromatography and the number of infested kernels per unit weight (Wehling et al 1984).

Holmes (1980) described a direct fluorometric method for the determination of uric acid in flour. Although the lower limit of

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measurement reported was only 20 μg g^{-1}, a sensitivity considerably less than the 1 μg g^{-1} needed for the analysis of commercial grain samples (Wehling et al 1984), preliminary experiments in our laboratory indicated that greater sensitivity was possible. Because of the potential of fluorometry to combine sensitivity with simplicity, the present study was undertaken to evaluate the possibility of using it to determine uric acid in grain.

MATERIALS AND METHODS

Wheat Samples

Most wheat samples were hard red winter wheats grown in Kansas. The bulk of the studies was done with the hard red winter wheat cultivar Larned grown in 1985 and 1987 near Hutchinson and Hays, KS. These were sound, uninfested samples selected as typical of wheats likely to be encountered in analyzing commercial samples. Hard red spring and soft red winter wheats also were examined. Samples with known concentrations of uric acid were prepared by adding weighed amounts to weighed samples of ground, uninfested, sound wheat in Erlenmeyer flasks with screw caps and thoroughly mixing the contents.

Apparatus and Reagents

Fluorescence measurements were made with a Farrand MK 2 spectrofluorometer (Farrand Optical Co., Inc., Valhalla, NY) equipped with a xenon arc light source and an X/Y recorder (model 715, MFE Corp.), which made possible automatic scanning and recording of both the excitation and emission spectra. Modified Czerny-Turner grating monochromators, each with a range of 200–700 nm, were used in both the exciter and analyzer. Wavelength accuracy was better than 1 nm with a readability of 0.25 nm. Fluorescence was detected by means of a photomultiplier tube. Fluorescence measurements were made in 45- × 10- × 10-mm quartz cells with the temperature controlled at 25.0° C by a circulating water bath. For recording the excitation and emission spectra of uric acid (Fig. 1), slit widths of the monochromators were set at 2.5 nm for the exciter and 5.0 nm for the analyzer. For all other measurements, slit widths were set at 5.0 nm for the exciter and 10.0 nm for the analyzer. The instrument was standardized against a 45- × 10- × 10-mm borosilicate glass standard. With the exciter wavelength set at 313 nm and the analyzer set at 351 nm, the fluorescence was set at 20–70 fluorescence units depending on the intensity of the light source and the requirements of the particular measurement.

Uric acid, 99%, was a crystalline product obtained from Sigma Chemical Co., St. Louis, MO. "Baker analyzed" disodium and trisodium phosphates, produced by J. T. Baker Chemical Co., Phillipsburg, NJ, and American Chemical Society (ACS) reagent-grade boric acid from Matheson, Coleman, and Bell were used to prepare buffers. Sodium tungstate was an ACS reagent-grade chemical from Fisher Scientific Co., Pittsburgh, PA, and uricase, a lyophilized powder from *Candida utilis* with an activity of 4.5 units per milligram, was obtained from Sigma Chemical Co. All other chemicals were ACS reagent grade or better.

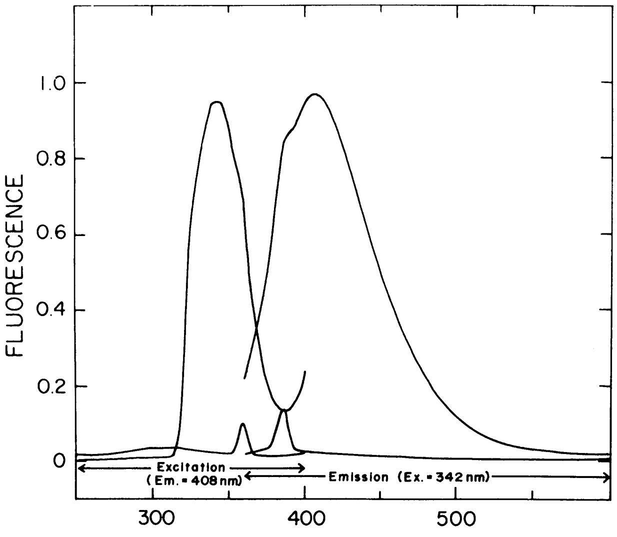

Fig. 1. Fluorescence spectra of uric acid in 0.20*M* sodium phosphate, pH 12.3. The upper curves represent the excitation (Ex.) and emission (Em.) spectra of uric acid, and the lower curves show the spectra of the solvent.

The solid-phase extraction tubes were obtained from Supelco Inc., Bellefonte, PA, Analytichem International, Harbor City, CA, J. T. Baker Chemical Co., and Waters Associates, Milford, MA. W-Porex SP300 was a product of Phenomenex Inc., Torrance, CA. Millex-PF 0.8-μm filter units were from Millipore Corp., Bedford, MA, and Diaflo membrane ultrafilters were from Amicon, a division of W. R. Grace & Co., Danvers, MA.

Extraction and Fluorescence Measurements

Grain samples were ground in a Wiley laboratory mill with a 10-mesh (U.S.) screen (2-mm openings) (intermediate model, Thomas Scientific, Swedesboro, NJ). A 1.0000-g sample was placed in a 50-ml round-bottom flask fitted with a ground-glass stopper, and 10.0 or 15.0 ml of 0.020*M* sodium borate buffer, pH 8.7, was added. The uric acid then was extracted by shaking it for 10.0 min at maximum speed (153 rpm) in a reciprocating shaker at room temperature. Suspended matter was separated by centrifugation at 27,000 × *g* for 10.0 min, and the supernate was saved for analysis. Early experiments showed that samples could be clarified by filtration through Whatman No. 2 filter paper followed by filtration through a Millex-PF 0.8-μm filter unit, but best results were obtained by centrifugation. Fluorescence measurements were made in 0.20*M* sodium phosphate buffer, pH 12.3.

Degradation of Uric Acid with Uricase

Uricase, 0.075 ml, with an activity of 1.41 unit ml^{-1}, in 0.020–0.050*M* sodium borate buffer, pH 8.7, was added to 0.810 ml of an extract, prepared and clarified as described above, of 1.0000 g of wheat containing uric acid in the same buffer. The solution was thoroughly mixed and allowed to react for 60–240 min or longer at room temperature. Before the fluorescence was read, sufficient 0.60*M* sodium phosphate buffer, pH 12.3, was added to give a final phosphate concentration of 0.20*M*. A blank was prepared from the same wheat extract with the uricase solution replaced with an equal volume of sodium borate buffer, pH 8.7. In some experiments, the concentration of enzyme was slightly increased without significantly altering the results.

Protein Precipitation with Tungstic Acid

A 1.0000-g sample of grain was extracted in 10.00 ml of sodium borate buffer and clarified by centrifugation as described above, and the pH of the clear extract was adjusted to 2.2 by adding 135 μl of 1.0*N* sulfuric acid. Solublized protein then was precipitated by adding 75 μl of 0.189*M* tungstic acid prepared by adding to a 10% (w/v) solution of disodium tungstate dihydrate an equal number of milliequivalents of 1.00*N* sulfuric acid. Before the fluorescence was read, the precipitate was removed by filtration through Whatman No. 2 filter paper.

Membrane Filtration

Several milliliters of an extract of a grain sample, prepared as described above by extraction into sodium borate buffer, pH 8.7, followed by centrifugation to remove suspended matter, was placed in an Amicon 8010 ultrafiltration cell equipped with a membrane filter. The cell then was pressurized with nitrogen, and the extract was filtered under pressure. Volume of the cell was 10 ml, permitting filtration of as little as 1.0 ml of solution, and its construction allowed the extract to be stirred continuously during filtration, thereby minimizing problems due to plugging of the membrane. Some samples were filtered in the pH 8.7 buffer used for extraction, and in other cases, for comparison, the pH of the extract was adjusted to 2.5–3.4 by adding hydrochloric or sulfuric acid before filtration, without any detectable effect on the amount of material removed by filtration.

Solid-Phase Extraction

For some samples, interfering polypeptides were removed by solid-phase extraction using Supelclean LC-SCX solid-phase extraction tubes (Supelco), which contained a cation exchanger with alkylsulfonyl groups bonded to 40-μm silica particles having 60-Å pores. After uric acid had been extracted from a 1.0000-g grain sample into 15.00 ml of 0.020*M* sodium borate buffer, pH 8.7, and the extract had been clarified by centrifugation as described above, the pH of the extract was adjusted to 3.5 by adding 270 μl of 0.50*N* hydrochloric acid. To two 3-ml LC-SCX tubes connected in series was added 1.50 ml of the acidified extract, and the extract was allowed to run slowly through the columns. When the level of liquid above the upper column just reached the top of the upper frit, an additional 0.50 ml of acidified extract was added, and 1.00 ml of eluate then was collected under vacuum. Immediately before the sample was added to the column pair, it was conditioned, in order, with 6.00 ml of methanol, 6.00 ml of double-deionized water, 6.00 ml of 0.10*N* hydrochloric acid, 6.00 ml of double-deionized water, and 2.50 ml of 0.020*M* borate buffer prepared and acidified in the same way as the buffer used to extract the sample.

RESULTS AND DISCUSSION

Excitation and Emission Spectra

Because uric acid is a purine with two ionizable hydrogens, its fluorescence would be expected to vary with pH. At pH 12.3 in 0.20*M* phosphate buffer, it gives the excitation and fluorescence spectra shown in Figure 1, with the excitation maximum at about 342 nm and an emission peak at about 408 nm.

In some of the earlier work, the fluorescence of uric acid was measured in acid solution, but more recent studies (Holmes 1980) have indicated that sensitivity is better under alkaline conditions. In alkaline solution, the fluorescence of uric acid is relatively independent of pH. From a pH of about 11 to 12.5, there is little or no variation in the fluorescence with pH. Thus, the necessary pH control can be achieved easily with relatively dilute phosphate buffer.

Linearity and Sensitivity

Over the range of 0–20 ppm, a calibration curve for the fluorescence of uric acid (Fig. 2) yields a straight line with a coefficient of correlation between the *x* and *y* variables, in this case, of 0.9998. The standard error of estimate of fluorescence with respect to parts per million uric acid, which was too small to be represented graphically in the figure, was only 0.0019 fluorescence units under the experimental conditions. Linear range extended to several hundred parts per million, well in excess of the concentration likely to be encountered in extracts of grain samples.

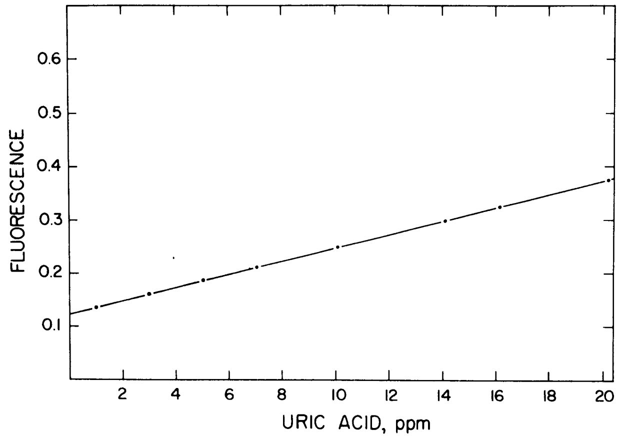

Fig. 2. Fluorescence of uric acid at 408 nm as a function of concentration in 0.20*M* sodium phosphate, pH 12.3, with excitation at 342 nm.

Because of the good precision, as indicated by the low standard error of estimate, analyzing for uric acid in the range of tenths of a part per million should be possible, a sensitivity sufficient to determine the levels commonly encountered in commercial grain samples. Of course, steps can be taken to further increase sensitivity, if desired. The most obvious step would be to use a more intense light source such as could be obtained with a xenon-mercury lamp by exploiting the mercury lines at 334 or 367 nm. Sensitivity also may be improved by lowering the sample temperature or by increasing the sensitivity of the photomultiplier, either by lowering its temperature to increase the signal-to-noise ratio or by selecting a more sensitive photomultiplier tube.

Background Fluorescence

The spectrum of an extract of an uninfested wheat sample shows a relatively high level of background fluorescence. In Figure 3, the middle curve shows the spectrum of an extract of uninfested, sound wheat in 0.20*M* sodium phosphate buffer, pH 12.3, and the upper curve gives the spectrum of a duplicate extract of the same sample containing 101 ppm of added uric acid. Something extracted from the wheat gave a very high level of background fluorescence at 408 nm, where the uric acid peak is. Preliminary experiments with corn showed a similar background fluorescence. Because of the high level of interfering fluorescence, the fluorometric analysis of uric acid in grain would require some method for correcting for or eliminating the background fluorescence.

Addition of Uricase

Uric acid undergoes an enzyme-catalyzed reaction that is sometimes used for its analysis. Under the influence of the enzyme uricase, uric acid is oxidized, with cleavage of the six-membered

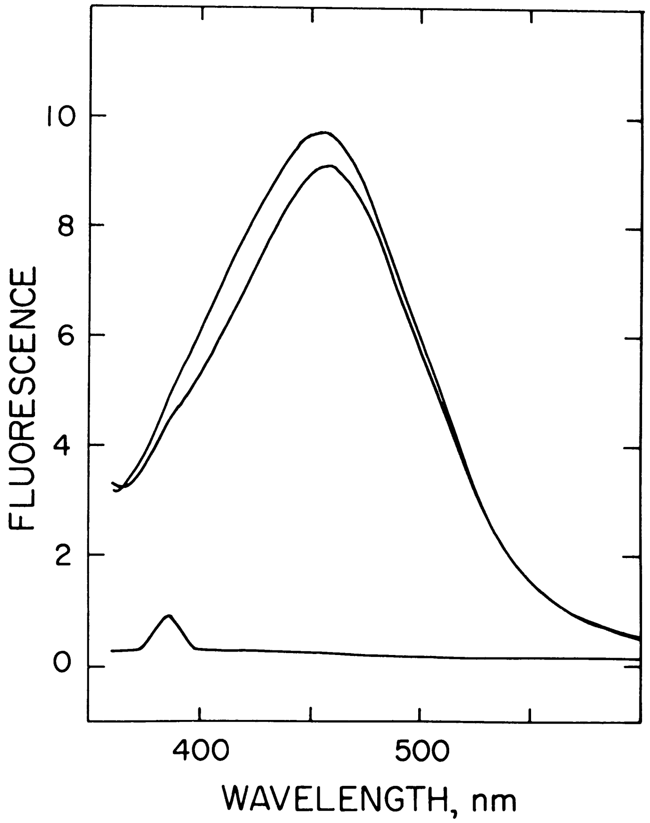

Fig. 3. Fluorescence spectrum in 0.20*M* sodium phosphate, pH 12.3, with excitation at 342 nm, of an extract of wheat cultivar Larned (1.0000 g/25.00 ml) with (upper curve) and without (middle curve) the addition of 101 ppm of uric acid. The lower curve shows the spectrum of the solvent.

ring to yield allantoin. Because allantoin is not fluorescent under the analysis conditions, the possibility existed that the level of background fluorescence could be determined after the destruction of uric acid by the addition of uricase. Because its pH optimum of 9.0 coincides with that recommended for the extraction of uric acid from grain (Wehling et al 1984), the use of uricase would be experimentally convenient.

As shown in Figure 4, the addition of uricase to an uninfested extract of Larned wheat containing a high level of added uric acid (101 ppm) did result in a reduced fluorescence. Correction for the background fluorescence, however, did not appear to be feasible. Although the uricase-catalyzed destruction of uric acid could be demonstrated easily, analyses for uric acid after correction for the background fluorescence were consistently low. The inaccuracy appeared to be a result of quenching by the compounds responsible for the background fluorescence at the high concentrations of extract needed to give the required sensitivity for uric acid.

Removal of Background Fluorescence

Because efforts to correct for the background fluorescence were unsuccessful, a method was needed for removal of the interference. This led to a series of studies to determine the cause of the background fluorescence and to devise a method for its elimination. For the fluorescence spectra shown in Figure 1, the excitation and emission wavelengths were set at the maxima for uric acid. The excitation and emission spectra of the background fluorescence of an uninfested sample of sound Larned wheat are shown in Figure 5, with the excitation and emission wavelengths set, in this case, at the correct maxima for the background fluorescence. The excitation maximum of the background fluorescence was at about 290 nm, and the emission peak was at about 354 nm. Because these maxima coincide with the values expected for proteins and peptides containing tryptophan, they effectively identify these polypeptides as the cause of the interfering fluorescence. Under the basic conditions (pH = ~9) used to extract uric acid from wheat, proteins representing all of the major classes—albumins, globulins, gliadins, and glutenins, as well as peptides and minor functional and structural proteins—would be extracted. Under the same conditions, analogous proteins and peptides would be extracted from corn and other grains.

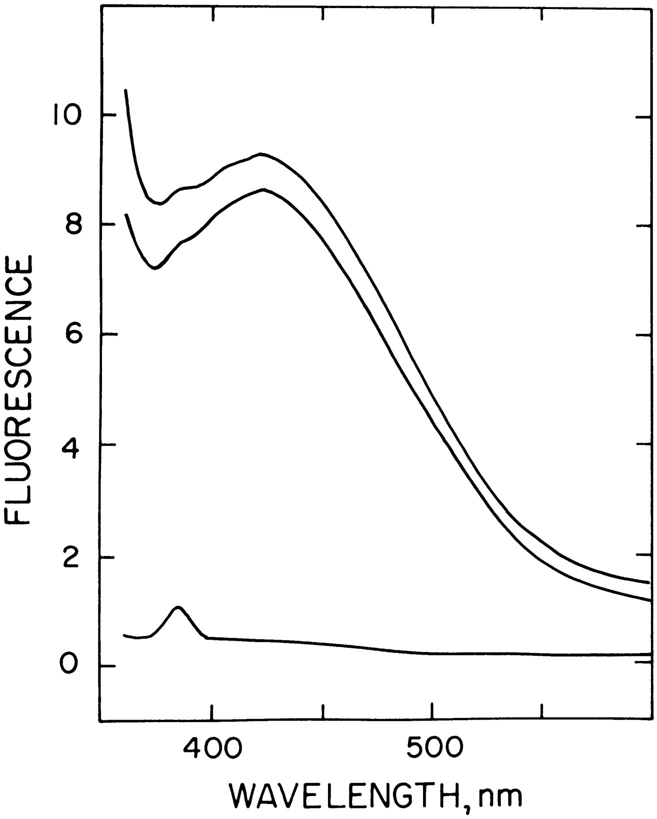

Fig. 4. Fluorescence spectrum, with excitation at 342 nm, of an extract of wheat cultivar Larned containing 101 ppm of added uric acid before (upper curve) and after (middle curve) the addition of uricase. The lower curve shows the spectrum of the solvent. A 1.0000-g wheat sample was extracted into 15.00 ml of sodium borate, pH 8.7, and the spectra were recorded in sodium phosphate, pH 12.3.

The problem of eliminating the background fluoroscence, then, becomes one of finding a way to remove the interfering tryptophan-containing polypeptides. Three methods were studied: use of protein-precipitating reagents, membrane filtration, and use of solid-phase extraction to selectively remove the polypeptides while allowing the uric acid to pass. Each method has been used to remove interfering protein in published analytical procedures. The requirements in this case, however, were demanding because of the need to remove a large amount of polypeptide in the presence of a few parts per million uric acid.

Protein precipitation. In evaluating the effectiveness of protein-precipitating reagents for removing the interference, best results were obtained with tungstic acid. Based on the fluorescence at 354 nm when excited at 290 nm, about one half of the interfering polypeptide was removed by the procedure described. Increasing the concentration of tungstic acid by adding more reagent did not result in the formation of additional precipitate. Although an appreciable portion of the interfering polypeptide was removed, the results indicate that, by itself, use of a protein-precipitating reagent would not eliminate the background fluorescence.

Membrane filtration. The membrane filters evaluated were made of cellulosic materials having low protein-binding properties and recommended for applications where maximum solute recovery is important. With the apparatus used, filtration was both rapid and convenient. Nominal molecular-weight cutoff values for the membranes tested were 500, 1,000, and 5,000. The amount of interfering polypeptide passing through a membrane was estimated by measuring the fluorescence at 354 nm both before and after filtration with excitation at 290 nm.

Filtration through the membrane with a molecular-weight

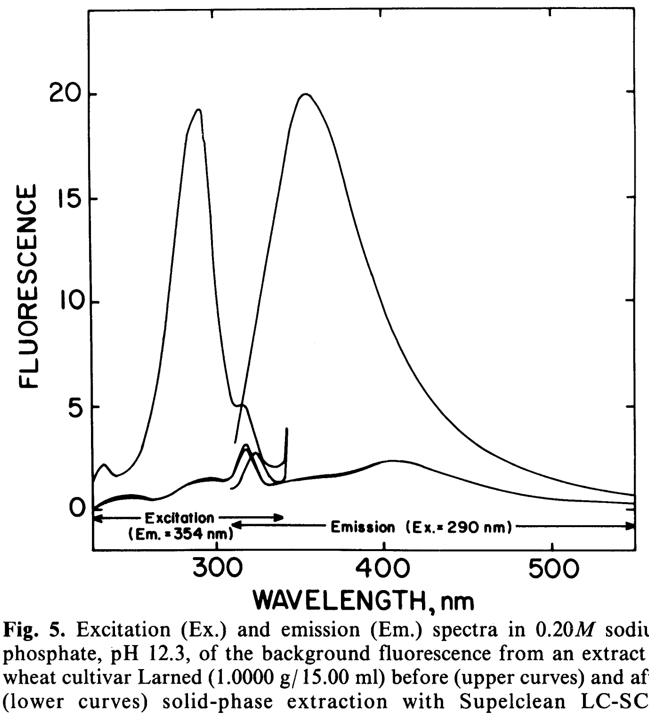

Fig. 5. Excitation (Ex.) and emission (Em.) spectra in 0.20M sodium phosphate, pH 12.3, of the background fluorescence from an extract of wheat cultivar Larned (1.0000 g/15.00 ml) before (upper curves) and after (lower curves) solid-phase extraction with Supelclean LC-SCX. Superimposed on the lower curves are the spectra of the solvent only. Before recording the fluorescence, both the extract and the treated extract were adjusted to 0.20M with respect to phosphate by adding 0.50 ml of 0.60M sodium phosphate, pH 12.6, to 1.00 ml of extract. After this adjustment, to keep the readings on scale, 1.00 ml of the untreated extract was diluted with 10.00 ml of 0.20M sodium phosphate, pH 12.3.

cutoff of 1,000 removed about 75% of the interfering polypeptide, whereas slightly less than 70% was removed by the membrane with a molecular-weight cutoff value of 5,000. Approximately 85% was removed by the membrane having a molecular-weight cutoff of 500. With this membrane, the addition of tungstic acid reagent before filtration did not remove additional interfering polypeptide, indicating that the protein-precipitating reagents are probably effective in removing only the relatively high-molecular-weight polypeptides. The membrane experiments clearly demonstrate that, although the bulk of the background fluorescence is from polypeptides having molecular weights above 5,000, a significant part is due, in addition to any free tryptophan, to peptides having molecular weights below 500. Because membranes with molecular-weight cutoff values of less than 500 are not available, membrane filtration by itself would not eliminate the interfering fluorescence. The membrane-filtration procedure was much more effective, however, than the addition of tungstic acid reagent.

Solid-phase extraction. A number of solid-phase extraction tubes were evaluated for removal of the polypeptides responsible for the interfering fluorescence. Tubes containing cation exchangers were Bond Elut SCX from Analytichem, with benzenesulfonylpropyl groups; Supelclean LC-SCX from Supelco, with 3-propylsulfonyl groups; and Baker-10 SPE WP COOH from J. T. Baker Chemical Co., with carboxyl groups. The first two tubes were 3 ml and had strong cation-exchange groups bonded to 40-μm silica particles with 60-Å pores, and the third tube was 6 ml and had weak cation-exchange groups bonded to 40-μm silica particles with 250-Å pores. In addition, 3-ml tubes were prepared containing W Porex SP300 from Phenomenex, with benzenesulfonylpropyl groups bonded to 10-μm silica particles with 300-Å pores. Also evaluated were 3-ml Supelclean LC-SAX tubes from Supelco, containing an anion exchanger with 3-quarternarypropyl ammonium groups bonded to 40-μm silica particles with 60-Å pores and C_{18} Sep-Pak cartridges from Waters Associates, which contained octadecyl groups for hydrophobic adsorption.

Best results were obtained with the cation exchangers in general and specifically with Supelclean LC-SCX, which contained alkylsulfonic acid groups. As shown in Figure 5, use of two of these tubes in series removed essentially all of the interfering fluorescence. The upper curves show the excitation and emission spectra of the sample, diluted 1:11 to keep the readings on scale, and the lower curves show the spectra of the sample after passage through the cation exchanger. The spectra of the sample after passage through the exchanger are virtually indistinguishable from those of the solvent, showing an essentially quantitative removal of the interfering proteins and peptides. To obtain a sufficient volume of extract for making a fluorescence measurement in the 1-cm cells used, two 3-ml Supelclean LC-SCX extraction tubes in series were needed for quantitative removal of the interfering polypeptides. Use of a smaller cell should allow quantitative removal with only a single LC-SCX extraction tube.

Because uric acid is not ionized at the pH of the sample during passage through the solid-phase extraction tubes to remove the interfering proteins and peptides, it would not be retained by the sulfonic acid cation exchanger, and its concentration in the solution should not change. Preliminary experiments using a single LC-SCX tube, which was almost as efficient as the two tubes in series for removing the interfering fluorescence, did not indicate any loss of uric acid.

Easier access of larger molecules to the exchange sites should make a cation exchanger with 300-Å pores better for removal of protein than one with 60-Å pores, but W Porex SP300, which has 300-Å pores, was not as effective as Supelclean LC-SCX, with 60-Å pores. Failure of the W Porex SP300 to work as well as the LC-SCX may have been due to its small particle size (10 μm) and the resulting low flow rate. The Baker-10 SPE WP COOH, which has 250-Å pores, was probably less effective than the Supelclean LC-SCX simply because it was a weak rather than a strong ion exchanger with the requirement that it be used at a higher pH where cation exchange is less effective. Although it has only half the exchange capacity (3 vs. 6 meq g^{-1}), the Supelclean LC-SCX worked better than the Bond Elut SCX, perhaps indicating a real difference between the aliphatic and aromatic sulfonic acid exchange groups but possibly reflecting only differences in manufacturing parameters. The Supelclean LC-SAX, a quarternary ammonium anion exchanger that required a different protocol with the uric acid adsorbed first on the column and then eluted after a pH adjustment, was considerably less effective than the cation exchangers, as was the C_{18} Sep-Pack cartridge.

CONCLUSIONS

Results indicate that fluorometry can be used to determine uric acid in grain with sufficient sensitivity to measure contamination or monitor insect infestation at levels commonly encountered in commerce. Application of the procedure requires the removal of interfering fluorescence, which was shown to be due to the presence in grain extracts of tryptophan-containing proteins and peptides. Although only a limited number of samples was examined, these proteins and peptides are known to be present in all wheats, and analogous proteins and peptides are found in other grains, including corn, barley, rye, oats, and rice. Quantitative removal of the interfering fluorescence was achieved by solid-phase extraction using 3-propylsulfonyl groups chemically bonded to 40-μm silica particles. Preliminary experiments showed that uric acid could be recovered from samples with added uric acid.

In the present study, procedures developed after careful experimentation by other workers for the extraction and recovery of uric acid from grain were used with only minor modification. Therefore, the amount of uric acid extracted from samples with added uric acid should not differ from that extracted from insect-infested samples containing equal concentrations. Because of the inherent advantages of fluorometry, removal of the interfering fluorescence may make it an attractive technique compared with other methods for the determination of uric acid in grain.

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