aged coffee industry, to give one example, our new procedure, as reported above, may prove of significant practical interest. To contrast it with a current situation, it should be mentioned that, most commonly, the available methods for assessing coffee adulteration fall, essentially, in two categories. To one of them belong those based on rather expensive and complex identification, and quantification, of some chemical constituents inequivocally associated with the adulterant only, and/or based on the search for noticeable changes in the relative content of some specific coffee related chemical constituents as compared to known values for pure coffee standards. To the other category belong the more popular, but rather coarse (low resolution), practice of directly confronting microscope-aided observations of equivalent samples of pure and adulterated powdered coffee samples (in the case of parchment this process might eventually allow for some kind of mechanical separation of the adulterant). Once some difference in texture is observed, the process is often followed by the evaluation of water-soluble solids obtained by strong extraction from coffee brewed by using the mixture under analysis. The residual content after a given amount of the beverage is evaporated is measured and compared against a similarly prepared sample from the beverage made with pure coffee. Aside from the inability of the method to tell apart different adulterants, all such methods suffer from rather poor resolution, not to mention the fact that marked alterations in the residue can be brought about by other sources intervening in the process (Cortez, 1983). Therefore, it does seem that the present method of photoacoustic evaluation of solid mixtures provides an adequate alternative for use in the powdered coffee industry, as well as in other similar food industries, one which compares quite favorably with other available methods. Needless to say, the same procedure applies to the determination of relative concentrations of any kind of solid (powdered) multicomponent mixture provided the aforementioned conditions are obtained.

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High-Performance Liquid Chromatographic Analysis of Fenridazon-potassium in Wheat Grain and Straw

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A high-performance liquid chromatographic residue analytical method is described for the analysis in wheat grain and straw of the chemical hybridizing agent fenridazon-potassium, potassium 1-(4-chlorophenyl)-1,4-dihydro-6-methyl-4-oxopyridazine-3-carboxylate. The method employs mild alkaline extraction, copper-activated Chelex 100 column chromatography, and liquid–liquid partition. Quantitation is by ion-pair, reverse-phase liquid chromatography with UV detection at 285 nm. Recoveries averaged 87.4 ± 11.3% for wheat grain and 86.2 ± 16.8% for wheat straw. Method sensitivity is 0.05 and 0.10 ppm for grain and straw, respectively.

Fenridazon-potassium, potassium 1-(4-chlorophenyl)-1,4-dihydro-6-methyl-4-oxopyridazine-3-carboxylate, is a wheat hydridizing agent. The hydridizing agent inhibits wheat pollen formation while maintaining the normal fertility of the female portion of the wheat flower.

Metabolism studies in wheat utilizing radioisotopically labeled fenridazon-potassium have demonstrated that residue resulting from use is the parent compound (Fisher and Peirson, 1978). Residue levels of field samples are greater than 0.10 ppm for wheat grain and straw. Therefore, we developed a highly sensitive residue analytical method that determines the presence of the hybridization agent in wheat.

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Samples are homogenized and extracted with petroleum ether to remove lipophilic constituents. Fenridazon-potassium is removed from the wheat grain or straw matrix by sodium bicarbonate extraction and separated from coextractives by column chromatography through copper-activated Chelex 100 chelating resin and liquid–liquid solvent partition. Quantitation is by ion-pair, reverse-phase high-performance liquid chromatography (HPLC) at 50 °C with UV detection at 285 nm. Calibration is performed by peak height using external standards. The limit of quantitation for wheat grain and straw is 0.05 and 0.10 ppm, respectively.

EXPERIMENTAL SECTION

Chemicals and Reagents. All organic solvents were HPLC grade from Burdick and Jackson. Ammonium hydroxide and hydrochloric acid were Mallinckrodt reagent

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grade. Water was HPLC grade from a Milli-Q purification system. Chelex 100 chelating resin, dry mesh 50–100, was purchased from Bio-Rad Laboratories. PIC A ion-pair reagent was from Waters. All other chemicals were Baker reagent grade.

Fenridazon-potassium reference standard (99.5%) was synthesized and purified at the Rohm and Haas Research Division Laboratories, Spring House, PA.

Standard solutions were prepared by serial dilution of a 200-ppm stock solution of fenridazon-potassium in the HPLC mobile phase.

The HPLC mobile phase was prepared by adding one vial of Waters PIC A reagent (15 mL) to a 1-L solution containing 30 mL of tetrahydrofuran, 10 mL of methanol, and 960 mL of water. The solution was filtered through a Millipore 0.45-μm HA filter. The HPLC mobile phase prepared in this way contained 0.005 M tetrabutylammonium phosphate at pH 7.5, phosphate buffered.

Copper-activated Chelex 100 resin was prepared in the following manner: 600 g of Chelex 100 was placed in a 2-L Erlenmeyer flask containing 1 L of distilled water, swirled gently, allowed to settle for 30 s, and decanted to remove the fine particulates. Fifty milliliters of a saturated aqueous cupric acetate solution was added to the remaining resin, swirled gently, allowed to settle for 2 min, and decanted. This process was repeated to ensure complete chelation of copper to the resin. The resin was washed twice with 1-L portions of 0.1 N sodium bicarbonate prior to packing the columns.

Equipment. Column chromatography was performed with Fischer and Porter 2.5 cm × 25 cm glass columns equipped with a 500-mL reservoir and a Teflon needle valve delivery fitting.

HPLC analyses were performed on a Du Pont Model 8800 liquid chromatography equipped with a Du Pont Model 851-401-901 column compartment and a Du Pont Model 834-001-901 automatic sampler.

The HPLC column was an Applied Science Adsorbosphere C_{18}, 4.6 mm i.d. × 250 cm, thermostated at 50 °C. The mobile phase, previously described, flowed at 2.0 mL/min. The injection volume was 100 μL. Detection was by UV at 285 nm.

Analytical Procedure. Bulk straw samples are homogenized before analysis by processing on a Wiley Mill Model 4.

A 20-g wheat grain sample or 10-g processed straw sample is homogenized in a Waring blender at high speed for 3 min. Petroleum ether (100 mL) is added and blended at low speed for 3 min. The mixture is vacuum filtered through a 150-mL funnel fitted with a coarse sintered glass frit. The petroleum ether filtrate is discarded, the filter cake is returned to the blender, and 70 mL of 0.1 N sodium bicarbonate solution for wheat grain or 120 mL of 0.1 N sodium bicarbonate solution for straw is added and blended at low speed for 5 min. The extraction mixture is transferred to a 250-mL centrifuge tube and centrifuged at 9000 rpm at 0 °C for 30 min.

The supernatant is decanted and saved. The pellet is returned to the blender by washes with 70 mL of 0.1 N $NaHCO_3$. Reextraction and centrifugation are performed exactly as before. Both supernatants are decanted and combined.

A 2.5 cm i.d. × 25 cm glass column is prepared by adding a 3.0-cm bed of washed Chelex 100 resin that has not been chelated with copper. This resin bed will retain copper ion that may elute from the chelated resin. The column is slurry packed to a total height of 20 cm with copper-activated Chelex 100. The column is equilibrated with 200 mL of 0.1 N $NaHCO_3$ at a flow rate of 3.5 mL/min by adjusting the needle delivery valve. After equilibration, the combined 0.1 N sodium bicarbonate extract is applied to the column. At a flow rate of 2.0 mL/min, the extract is allowed to elute to the top of the resin bed, and the eluate is discarded. The bed is washed with 40 mL of 0.1 N sodium bicarbonate. After the wash, the column is eluted with ammonium hydroxide. The last 20 mL of sodium bicarbonate wash and the 150 mL of 1.0 M ammonium hydroxide eluant are collected. Five grams of sodium chloride is dissolved in the eluant. The eluant is acidified to pH 2.0 with 12–15 mL of concentrated hydrochloric acid added dropwise. The extract is sonicated until evolution of carbon dioxide (as seen by bubbling) ceases.

The acidified extract is quantitatively transferred when cool to a 1000-mL separatory funnel containing 250 mL of fresh methylene chloride and partitioned. If an emulsion forms, 50 mL of methylene chloride is added to aid in phase separation. The lower methylene chloride phase is collected. The aqueous phase is reextracted with 250 mL of methylene chloride, and the methylene chloride extracts are combined in a 1-L round-bottomed flask.

The combined volume of the methylene chloride extracts is reduced to ca. 30 mL by rotary evaporation at 40 °C under diminished pressure. The extracts are quantitatively transferred to a 100-mL round-bottomed flask, 10 mL of methanol is added, and the mixture is evaporated to dryness by rotary evaporation at 40 °C under diminished pressure. The sample is dissolved in 10 mL of the HPLC mobile phase and sonicated to ensure complete dissolution. The solution is filtered through a 0.45-μm Gelman Acrodisc sterile filter, Model 4184, into automatic sampler vials for high-performance liquid chromatography. The HPLC column is equilibrated with the mobile phase at a flow rate of 2.0 mL/min for 40 min. One-hundred microliters of the sample solution and standards is injected. Quantitation is performed by peak height using external standards. Residues are quantitated by comparing the chromatographic peak heights of the fenridazon-tetrabutylammonium salt in the sample solution with the standard curve constructed of the peak heights for fenridazon-tetrabutylammonium salt.

RESULTS AND DISCUSSION

Chelex 100 is a styrene–divinylbenzene copolymer containing paired imino diacetate ions that function as chelating groups in binding metal ions. Chelex 100 is used to scavenge trace metals from water (Figura and McDuffie, 1977), food digests (Baetz and Kenner, 1975), and reagents (Poyer and McCay, 1971). The resin exhibits a very high selectivity for divalent copper ions (Bio-Rad Laboratories, 1981). This Chelex 100–copper complex has been used in the separation of amino acids from seawater (Siege and Degens, 1966) and urine (Buist and O'Brien, 1967), in the spearation of nucleosides, nucleotides, and nucleic acid bases (Goldstein, 1967), and in high-performance liquid chromatography of amino acids (Szczepaniak and Ciszewska, 1982). Fenridazon-potassium exhibits a high affinity for this copper–Chelex 100 complex and is quantitatively removed from the 0.1 N sodium bicarbonate extract, resulting in a significant purification. Ammonium hydroxide with high affinity toward divalent copper ions is employed to elute fenridazon-potassium from the copper–Chelex 100 matrix.

Representative chromatograms for the determination of fenridazon-potassium in wheat grain are illustrated in Figure 1. Figure 2 shows representative chromatograms of straw. The chromatograms demonstrate that fenrida-

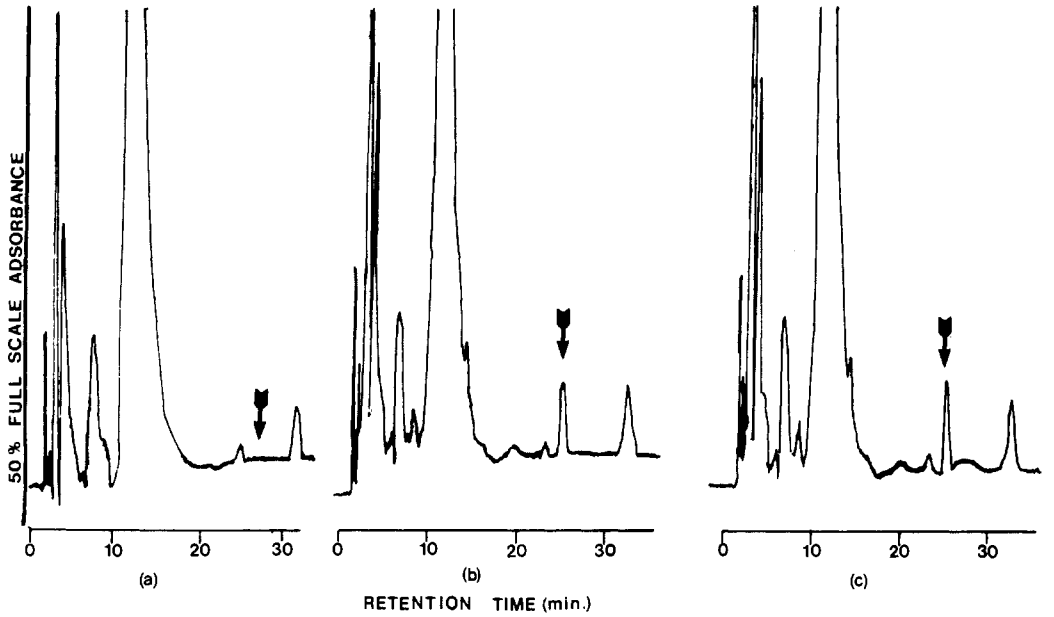

Figure 1. Fenridazon-potassium in wheat grain: (a) control wheat grain; (b) fortification 0.05 ppm; (c) treated sample 0.07 ppm.

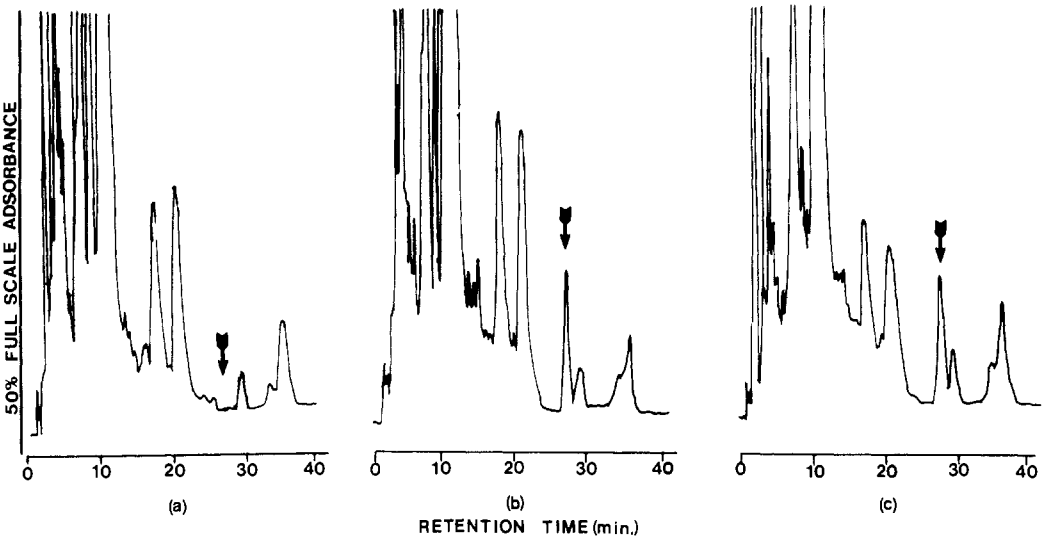

Figure 2. Fenridazon-potassium in wheat straw: (a) control wheat straw; (b) fortified wheat straw 0.15 ppm; (c) treated sample 0.15 ppm.

zon-potassium is adequately resolved from any interfering components.

Standard curves were linear with a zero intercept for injections of up to 1.0 μg. The analytical sensitivity was typically 1.5 mm/ng at 0.005 AUFS.

Controls used were the female donor of the hybrid wheat. No true controls exist for treated samples as the hybrid is unique and must be exposed to fenridazon-potassium to exist.

Recovery efficiencies were determined by fortifying control wheat grain before the initial petroleum ether extraction with fenridazon-potassium at concentrations ranging from 0.05 to 10.5 ppm. Control straw samples were fortified from 0.10 to 4.0 ppm. Recovery data are summarized in Table I. Recoveries for wheat grain averaged 87.4 ± 11.3%. Recoveries for straw averaged 86.2 ± 16.8%.

The limit of quantitation of the method was evaluated by actual recoveries of fortified controls at the lower limits of sensitivity. The limit of quantitation for wheat grain proved to be 0.05 ppm, and the limit of quantitation for straw proved to be 0.10 ppm.

Table I. Fenridazon-potassium Recovery Data for Wheat Grain and Straw

sample type	fortification level, ppm	av % recovery (no. of fortifications)	recovery range, %
grain	10.5	89.8 (4)	71–114
	5.0	97.5 (2)	93–102
	4.0	86.7 (3)	75–94
	2.5	83.8 (4)	75–90
	2.0	93.5 (4)	80–105
	1.0	87.3 (7)	80–110
	0.5	85.0 (6)	71–98
	0.2	87.2 (5)	79–111
	0.05	83.0 (6)	72–104
	average grain recovery = 87.4 ± 11.3%; range = 71–114%		
straw	4.0	69.0 (2)	69–69
	2.0	85.3 (3)	70–100
	1.0	89.5 (2)	86–93
	0.5	90.3 (3)	69–109
	0.2	78.3 (3)	70–92
	0.1	86.3 (3)	59–108
	0.05	102.0 (3)	86–114
	average straw recovery = 86.2 ± 16.8%; range = 59–114%		

The fenridazon-potassium HPLC residue analytical method is reproducible and sensitive for wheat grain and straw.

Registry No. Fenridazon-potassium, 83588-43-6.

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Lipid Class and Fatty Acid Compositions of Young *Amaranthus gangeticus* L. Leaves

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The *Amaranthus gangeticus* (Amaranthaceae) leaves yielded on extraction with chloroform–methanol 10.6% lipids (dry weight), which were separated into nonpolar lipids (53.6%), glycolipids (33.8%), and phospholipids (12.6%) by silicic acid column chromatography. These were further fractionated into subclasses by thin-layer chromatography. The nonpolar lipids were made up (wt %) of pigments (8.1), hydrocarbons (4.9), ester waxes (1.8), fatty acid methyl esters (2.7), triacylglycerols (6.4), fatty acids (5.6), diacylglycerols (5.6), sterols (9.3), monoacylglycerols (4.7), and unidentified components (4.5). The glycolipids comprised (wt %) monogalactosyl diglycerides (15.6), steryl glycosides (4.1), cerebrosides (6.8), and digalactosyl diglycerides (7.3). The phospholipids consisted (wt %) of cardiolipin (2.0), phosphatidylglycerol (3.1), phosphatidylethanolamine (3.2), phosphatidylinositol (1.7), and phosphatidylcholine (2.6). The usual fatty acids were found in varying concentrations in different lipid classes. *trans*-3-Hexadecenoic acid amounted to 12.3% in phosphatidylglycerol fatty acids.

Amaranthus gangeticus Linn. (Amaranthaceae) is a widely cultivated plant. The leaves of the young plants are used in culinary preparations all over India. There is no information on the lipids. This paper reports for the first time the nature and contents of various lipid classes as well as the constituent fatty acids present in a typical sample of young leaves normally used in culinary preparations.

EXPERIMENTAL SECTION

Extraction and Purification of the Lipids. *A. gangeticus* plants (174) were uprooted at random on the 20th day after sowing the seeds in a prepared plot. The leaves were separated. Moisture content was determined on a portion of pooled leaves. The remaining leaves were weighed and dipped in hot water to inactivate the leaf lipases (Haverkate and Van Deenen, 1965). The lipids were extracted and purified according to Folch et al. (1957). An aliquot of the chloroform fraction was used for determining the lipid content.

Separation of Nonpolar and Polar Lipid Classes. The leaf lipids were separated on a silicic acid column by using chloroform, acetone, and methanol (Carroll, 1976). The chloroform eluate contained the nonpolar lipids from which pigments were separated by passing through a charcoal–Celite column (Khor, 1979). The acetone fraction contained glycolipids and the methanol fraction phospholipids.

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Table I. Lipid Class Composition (Weight Percent) of Young *A. gangeticus* Leaves[a,b]

nonpolar lipids (53.6)	
pigments	8.1
hydrocarbons	4.9
ester waxes	1.8
fatty acid methyl esters	2.7
triacylglycerols	6.4
fatty acids	5.6
diacylglycerols	5.6
sterols	9.3
monoacylglycerols	4.7
unidentified	4.5
glycolipids (33.8)	
monogalactosyl diglycerides	15.6
steryl glycosides	4.1
cerebrosides	6.8
digalactosyl diglycerides	7.3
phospholipids (12.6)	
cardiolipin	2.0
phosphatidylglycerol	3.1
phosphatidylethanolamine	3.2
phosphatidylcholine	2.6
phosphatidylinositol	1.7

[a] Tetradecane, myristyl palmitate, methyl stearate, sesame oil, commercial monoglycerides, oleic acid, and stigmasterol were used for identification of nonpolar lipids. Authentic glycolipid and phospholipid classes, and sulfolipids that were not detected, were used as reference. [b] Overall recovery of total lipids after column chromatography followed by TLC was 92.3%.

Fractionation of Lipid Classes. Preparative thin-layer chromatography (TLC) on 0.8-mm layers of silica gel G using a solvent system of petroleum ether (40–60 °C)–diethyl ether–acetic acid (90:10:1 v/v) (Mangold and

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