20, 117121-50-3; 21, 117121-51-4; 22, 117121-52-5; 23, 117121-53-6; 24, 117121-54-7; 25, 117121-55-8; 26, 117121-56-9; 27, 117121-57-0; 28, 117121-58-1; 29, 117121-59-2; 30, 117121-60-5; 31, 117121-61-6; 32, 117121-62-7; 33, 117121-63-8; 34, 117121-64-9.

LITERATURE CITED

Bishop, N. I. Influence of the herbicide, DCMU, on the oxygen-evolving system of photosynthesis. *Biochim. Biophys. Acta* **1958**, *27*, 205–206.

Briggs, G. G. Theoretical and experimental relationships between soil adsorption, octanol-water partition coefficients, water solubilities, bioconcentration factors and parachor. *J. Agric. Food Chem.* **1981**, *29*, 1050–1059.

Camilleri, P.; Bowyer, J. R.; Gilkerson, T.; Odell, B.; Weaver, R. C. Structure–activity relationships in the Hill inhibitory activity of substituted phenylureas. *J. Agric. Food Chem.* **1987**, *35*, 479–483.

Hansch, C. Quantitative approach to biochemical structure–activity relations. *Acc. Chem. Res.* **1969**, *2*, 232–239.

Kakkis, E.; Palmire, V. C.; Strong, C. D.; Bertsch, W.; Hansch, C.; Schirmer, U. Quantitative structure–activity relationships in the inhibition of Photosystem II in chloroplasts by phenylureas. *J. Agric. Food Chem.* **1984**, *32*, 133–144.

Mitsutake, K.; Iwamura, H.; Schimizu, R.; Fujita, T. Quantitative structure–activity relationships of Photosystem II inhibitors in chloroplasts and its link to herbicidal action. *J. Agric. Food Chem.* **1986**, *34*, 725.

Seewald, I.; Michael, H. J.; Klepel, M.; Held, P.; Ohmann, E.; Barth, A.; Metzger, U. Quantitative Structure-Activity Analysis. *Akad. Verlag Berlin* **1978**, 77.

Takemoto, I.; Yoshida, R.; Sumida, S.; Kamoshita, K. Quantitative structure-activity relationships of substituted phenylureas on the Hill inhibitory activity. *J. Pestic. Sci.* **1984**, *9*, 517–521.

van den Berg, C.; Tipker, J. Quantitative structure-activity relationships of the inhibition of photosynthetic electron flow by substituted diphenyl ethers. *J. Pestic. Sci.* **1982**, *13*, 29–38.

van Rensen, J. J. S. Molecular mechanisms of herbicide action near Photosystem II. *Physiol. Plant.* **1982**, *54*, 515.

Verloop, A.; Hoogenstraaten, W.; Tipker, J. Development and application of new steric substituent parameters in drug design. *Drug Design*; Academic: New York, 1976; Vol. VII, pp 165–207.

Wessels, J. S. C.; van der Veen, R. Action of derivatives of phenylurethan and of 3-phenyl-1,1-dimethylurea in the Hill reaction. *Biochim. Biophys. Acta* **1956**, *19*, 548–549.

Received for review January 20, 1988. Accepted May 26, 1988.

Effect of Soil and Foliar Daminozide Applications on Residue Levels in Peanut

Craig S. Kvien,* Richard A. DePalma, Art R. Raczynski, and Lesle M. Hellmann

Daminozide is used in the Southeast United States to control excess peanut (*Arachis hypogaea* L.) vine growth. Two states now require processed food to meet a none detected level of daminozide by 1990. This study determined the within-plant concentration and location of daminozide residues resulting from soil carryover of daminozide and from foliar applications of daminozide. Applications of 1.43 kg ha^{-1} daminozide to the soil immediately before planting resulted in no residue in any plant part at harvest. Plants treated with either a single foliar application of 0.95 kg ha^{-1} at 42 days after planting (DAP) or 0.95 kg ha^{-1} at 42 DAP plus 0.48 kg ha^{-1} at 86 DAP had mature fruit residues of 0.27 and 4.5 ppm, respectively. Foliar-applied daminozide is translocated throughout the plant. Residues in the foliage are predictive of residues in the seed. Foliar samples taken before harvest can be used as a diagnostic tool to locate daminozide-treated plants.

One of the major uses of plant growth retarding chemicals in the Southeast United States is to control excess peanut (*Arachis hypogaea* L.) vine growth (N'Diaye, 1980). Since the peanut is a perennial (Hoehne, 1940) with an indeterminate fruit set pattern and season-long shoot growth, harvesting and plant disease problems often result from excessive vine growth.

To prevent excess vine, some peanut growers have applied the plant growth retarding chemical daminozide to their crop (Brown and Ethredge, 1974; N'Diaye, 1980; Ohaly, 1985; Kvien et al., 1987). Although daminozide is both xylem and phloem mobile, plant growth regulating activity is dependent on foliar absorption since daminozide is rapidly degraded in the soil (Rothenberger, 1964; Moore, 1968; Uniroyal, 1981).

Department of Agronomy, University of Georgia Coastal Plain Experiment Station, P.O. Box 748, Tifton, Georgia 31793 (C.S.K.), The Procter and Gamble Company, Winton Hill Technical Center, 6071 Center Hill Road, Cincinnati, Ohio 45224 (R.A.D., A.R.R.), and Hill Top Biolabs, Inc., P.O. Box 429501, Cincinnati, Ohio 45242 (L.M.H.).

Two states now require processed food to have a none detected level of daminozide by 1990. New analytical techniques have lowered detection limits from 0.1 ppm for apple products or 2 ppm for peanut products to 0.01 ppm for all food products (Wright, 1987; Conditt and Baumgardner, 1988).

The studies that determined daminozide was not carried over from one cropping season to the next were conducted at the 2 ppm detection limit (Rothenberger, 1964). The potential for planting peanut on land treated with daminozide the previous season exists. Therefore, it is important to know whether these peanuts will meet the lower daminozide residue requirement. This study was conducted to determine the within-plant concentration and location of daminozide residues resulting from soil carryover of daminozide and from foliar applications of daminozide.

MATERIALS AND METHODS

This greenhouse experiment was conducted in 4-L pots arranged in a randomized complete block design with five treatments, three replications, and six pots per replication–treatment combination (90 total pots). The five experimental treatments were as follows: (1) A control soil

0021-8561/89/1437-0200$01.50/0

Table I. Effect of Foliar- and Soil-Applied Daminozide on Residue Levels (ppm) in the Soil and Various Peanut Plant Tissues 115 Days after Planting (DAP)

	daminozide treatment, kg/ha	type of appl	time of appl	soil	root	shoot	fruit
1	control	no daminozide		<0.05[a]	<0.05	<0.05	<0.05
2	1.43	foliar-applied[b]	7 months before planting	<0.05	<0.05	<0.05	<0.05
3	1.43	soil-applied	4 h before planting	<0.05	<0.05	<0.05	<0.05
4	0.95	foliar-applied	42 DAP	<0.05	0.17	2.00	0.17
5	0.95 + 0.48	foliar-applied	42 DAP + 86 DAP	<0.05	0.61	8.00	4.50

[a] 0.05 ppm was the detection limit due to the 10-g sample size. [b] This treatment was foliar-applied to a field test plot of peanut. The crop (seed) was harvested, the vines were incorporated into the soil, and the soil was then gathered for the experiment 7 months later. No additional daminozide application was made.

had no applications of daminozide for at least 10 years and received no daminozide treatments during the experimental period. (2) This soil came from a field research site where peanut was treated with 0.95 kg ha^{-1} daminozide on 13 June 1986, and again with 0.48 kg ha^{-1} daminozide on 24 July 1986. Peanut seed were harvested from these plots on 15 Sept 1986 and the vines disked into the soil (typical of normal cultural practice). Soil samples for this treatment were collected on the same day as the test was planted, 25 Feb 1987. (3) Daminozide-free soil (same soil source as treatment 1) was treated with 1.43 kg ha^{-1} daminozide applied 4 h before planting. (4) One foliar daminozide (0.95 kg ha^{-1}) treatment was applied 8 April 1987 [42 days after planting (DAP)] to plants growing in daminozide-free soil (same soil source as treatment 1). (5) Two foliar daminozide treatments [0.95 kg ha^{-1} on 8 April 1987 (42 DAP) and 0.48 kg ha^{-1} on 22 May 1987 (86 DAP)] were applied to plants growing in daminozide-free soil (same soil source as treatment 1).

The Tifton loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudults) soil used in this study came from replicated 1986 peanut plot areas located on the University of Georgia Coastal Plain Experiment Station near Tifton, GA. The application rate of daminozide to the potted soil before planting was calculated on a surface area basis; the daminozide was then applied in 50 mL of water and immediately worked into the top 7 cm of the soil. Foliar treatments were applied to pots (arranged outside the greenhouse to simulate a field planting) with a precision field sprayer equipped with three D2-13 nozzles per row that delivered 170 L of water ha^{-1} (water/hectare). Soil moisture, fertility, insect and disease control levels, and practices were consistent with Georgia Cooperative Extension Service recommendations (Womack et al., 1981). Daminozide supplied by Uniroyal (Uniroyal Chemical Co. Inc., Middlebury, CT) was formulated as an 0.85 WP (wettable powder). The experiment was planted on 2 Feb 1987, using the cultivar Florunner. The seed were free of daminozide residue.

At 115 DAP (22 June 1987) and 137 DAP (the mature crop harvest date, 14 July 1987) soil and plant tissue (root, fruit, shoot) samples were taken from three pots of each replication and treatment combination. Samples were frozen at −10 °C, packed in dry ice, and express-shipped to The Procter and Gamble Co. in Cincinnati, OH, for analysis.

Samples were analyzed by the method of Conditt and Baumgardner (1988). This method hydrolyzes the daminozide in the presence of a strong base to form the unsymmetrical dimethylhydrazine (UDMH), which is distilled from the sample matrix. A stable derivative is formed by reacting UDMH salicylaldehyde dimethylhydrazone. This derivative is separated and quantified by GC/MS using selected ion monitoring of key ions in the fragmentation pattern. An internal standard, 4-nitroanisole, is used in the derivative step of the analysis to improve the precision of the GC/MS quantitation.

Calibration curves were distilled from a daminozide-free sample (treatment 1) matrix for each sample type (soil, root, shoot, mature fruit) to duplicate the sample matrix where possible. No daminozide residue was found in any of the plant parts from the untreated control except in the root when a 50-g sample was used. Since no daminozide was used in this treatment, this is probably an interference only seen at the 50-g sample weight. This response is so small that the confirmation ion in the selected ion monitoring mode could not be used to verify the authenticity of the compound because of the low intensity of the confirmation ion. Immature fruit sample size was limited; therefore, a daminozide-free peanut butter matrix was used for the immature fruit calibration curve.

At 115 DAP, sample weights of 10 g from each soil and plant part were analyzed. The calibration curves for the 115 DAP samples were 0.5, 1, and 2 ppm. The 10-g sample size of the 115 DAP sampling results in a detection limit of 0.05 ppm, and therefore results from this sampling are reported at a 0.05 ppm detection limit.

At 137 DAP, sample weights of 50 g from each soil, root, and shoot sample were used while 10 g was used for the fruit because of sample size limitations. The soil and plant parts were analyzed in triplicate. The calibration curves for the 137 DAP samples were 0.02, 0.1, 0.4, and 1 ppm. This calibration curve gave a positive response for daminozide in the root samples where a small response was found in the untreated control where no daminozide was used. Accurate detection above 0.01 ppm with the 50-g sample was possible. Therefore, the 137 DAP sampling is reported with a 0.01 ppm detection limit for the soil, root, and shoot. Sample size (10 g) again limited daminozide detection in the fruit tissue to 0.05 ppm.

Linear regression was used to construct the calibration curve. The ratio of the analyte to the internal standard was plotted against amount of analyte, and the correlation coefficient exceeded 0.97. Data from the experiment were analyzed as a randomized complete block design with use of the PROC GLM and PROC MEANS procedures of SAS (1985).

RESULTS

For the samples taken 115 DAP, no daminozide was found above the detection limit of 0.05 ppm in soil or plant tissues of the control or either of the daminozide treatments applied before the test peanut were planted (Table I). One 0.95 kg ha^{-1} foliar application of daminozide applied 42 DAP resulted in residue levels of <0.05 ppm in the soil and residues ranging from 0.17 to 2.0 ppm in the plant tissues (Table I). Foliar applications of 0.95 + 0.48 kg ha^{-1} daminozide, at 42 and 86 DAP respectively, resulted in residue levels ranging from <0.05 to 8.0 ppm (Table I).

For the samples taken 137 DAP, no daminozide was found above the detection limit of 0.01 ppm in the soil or

Table II. Effect of Foliar- and Soil-Applied Daminozide on Residue Levels (ppm) in the Soil and Various Peanut Plant Tissues 137 Days after Planting (DAP)

	daminozide treatment, kg/ha	type of appl	time of appl	soil	root	shoot	immature fruit	mature fruit
1	control	no daminozide		<0.01[a]	0.02[b]	<0.01	<0.01	<0.01
2	1.43	foliar-applied[c]	7 months before planting	<0.01	0.02	<0.01	<0.01	<0.01
3	1.43	soil-applied	4 h before planting	<0.01	0.10	<0.01	<0.01	<0.01
4	0.95	foliar-applied	42 DAP	<0.01	0.16 ± 0.06	0.64 ± 0.16	0.06 ± 0.01	0.27 ± 0.03
5	0.95 + 0.48	foliar-applied	42 DAP + 86 DAP	0.02[d]	0.36 ± 0.60	3.03 ± 0.60	1.10 ± 0.32	4.50 ± 0.61

[a] 0.05 and 0.01 ppm were the detection limits for the 10- and 50-g sample sizes, respectively. [b] Interference compounds in the root tissue resulted in false-positive readings of 0.02 ppm. [c] This treatment was foliar-applied to a field test plot of peanut. The crop (seed) was harvested, the vines were incorporated into the soil, and the soil was then gathered for the experiment 7 months later. No additional daminozide application was made. [d] The 0.02 ppm of daminozide found in the soil for this treatment could be due to small amounts of root in the soil.

shoot tissue of the control or either daminozide treatment applied before planting (Table II). Interfering compounds resulted in false-positive readings of 0.02 ppm in the root tissue of the control and both daminozide applications made before planting (Table II). One 0.95 kg ha^{-1} foliar application of daminozide made 42 DAP resulted in residue levels of <0.01 ppm in the soil and residues ranging from 0.06 to 0.64 ppm in the plant tissues (Table II). Foliar applications of 0.95 + 0.48 kg ha^{-1} daminozide, at 42 and 86 DAP, respectively, resulted in residue levels ranging from 0.02 to 4.5 ppm (Table II). The 0.02 ppm of daminozide found in the soil for this treatment could be due to small amounts of root in the soil.

DISCUSSION

Daminozide applied at 1.43 kg ha^{-1} to the soil immediately before planting represented a maximum soil residue. Daminozide was not detected in plants grown in this maximum residue soil. Rothenberger (1964) described daminozide as being rapidly bound to the soil and degraded. Uniroyal (1981) reported that daminozide is very mobile in sandy loam soils, microbial breakdown is rapid, and daminozide is not persistent in soil, with 50% of the material applied to representative soils dissipated within 1 week. We conclude that peanut planted in fields treated the previous year with daminozide will not have detectable residues at harvest.

Foliar-applied daminozide resulted in detectable daminozide levels in all plant parts at both the 115 and 137 DAP samplings (Tables I and II). Residues were generally higher in the 115 DAP sampling than in the 137 DAP sampling (Tables I and II). Differences in residues between sampling dates are probably due to further vegetative and reproductive growth (diluting residues), translocation of residues to developing fruit, and plant degradation of the residues.

Application of 0.95 kg ha^{-1} at 42 DAP followed by 0.48 kg ha^{-1} at 86 DAP resulted in mature fruit residues of 4.5 ppm compared with residues of 0.27 ppm from a single application of 0.95 kg ha^{-1} at 42 DAP (Table II). Peanut has an indeterminate fruiting pattern; the first fruit begins forming approximately 42 DAP, and new fruit continues to form throughout the season. Early (42 DAP) applications of daminozide will result in less residue in mature fruit than late-season applications for several reasons: (1) The plant has more time to break down the compound before harvest. (2) Very few fruit are present to act as sinks for the daminozide. (3) The plant canopy is smaller early in the season, resulting in less daminozide being intercepted by the foliage.

The daminozide use label for peanut allows up to 1.43 kg ha^{-1} applied to the crop, with the last application to be made no fewer than 30 days before harvest. Three maximum rate applications are listed on the label: (1) 0.95 kg ha^{-1} early followed by 0.43 kg ha^{-1} 6 weeks later (similar to our treatment 5); (2) three equal applications of 0.48 kg ha^{-1} made in early, middle, and late season; (3) six equal applications of 0.24 kg ha^{-1} 10–14 days apart starting 40 DAP (Uniroyal, 1988).

In our studies, an initial foliar application of 0.95 kg ha^{-1} followed by an additional treatment of 0.48 kg ha^{-1} resulted in a 17-fold increase in mature fruit residue levels when compared to the single 0.95 kg ha^{-1} treatment. We conclude that the more daminozide applied after fruit set, the greater the fruit residue levels will be. Therefore, residues will be higher with label option 3 than option 2 and option 1 will result in the lowest residue.

Our data indicate to us that daminozide applied to the foliage is translocated throughout the plant. Residues in the foliage are predictive of residues in the seed. Foliar samples taken several weeks before harvest can be used as a diagnostic tool to locate daminozide-treated plants.

ACKNOWLEDGMENT

The help of F. Barnes, M. Conditt, D. Hardy, and D. Osborne was appreciated. This work was supported by state and Hatch funds and grants from the Georgia Agricultural Commodity Commission for Peanut and the National Peanut Council.

Registry No. Daminozide, 1596-84-5.

LITERATURE CITED

Brown, R. H.; Ethredge, W. J. Effects of Succinic Acid 2,2-Dimethylhydrazide on Yield and Other Characteristics of Peanut Cultivars. *Peanut Sci.* **1974**, *1*, 20–23.

Conditt, M. K.; Baumgardner, J. R.; Hellmann, L. M. Gas Chromatographic/Mass Spectrometric Determination of Daminozide in High Protein Food Products. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 735–739.

Hoehne, F. C. Leguminosas-papilionadas genero *Arachis*. In *Flora Brasilica* Romiti & Lanzara: San Paulo, Brazil, 1940; Vol. 25 (II), p 122.

Kvien, C. S.; Csinos, A. S.; Ross, L. F.; Cokerton E. J.; Styer C. Diniconazole's Effect on Peanut (*Arachis hypogaea* L.) Growth and Development. *J. Plant Growth Reg.* **1987**, *6*, 233–244.

Moore, t. C. Translocation of the Growth Retardant N,N-Dimethylaminosuccinamic acid-14C (B-995-14). *Bot. Gaz.* **1968**, *129*, 280–285.

N'Diaye, O. Physiological Aspects of Peanut (*Arachis hypogaea* L.) Yield as Affected by Daminozide. Ph.D. Dissertation, The University of Florida, 1980.

Ohaly, J. Daminozide—A Growth-Regulator for Peanuts. *Phytoparasitica* **1985**, *13*, 236.

Rothenberger, R. R. Studies of the Translocation, Metabolism and Environmental Responses of Ornamental Plants to Chemical Growth Retardants. Ph.D Dissertation, The University of Missouri, 1964.

SAS Institute. *SAS User's Guide: Statistics*; SAS Institute: Cary, NC, 1985.

Uniroyal. Butanedioic acid mono (2,2-dimethylhydrazide). In *Plant Growth Regulator Handbook*; Plant Growth Regulator

Working Group: Longmont, CO, 1981.
Uniroyal. *Sample Labels*; Uniroyal Chemical: Middlebury, CT, 1988.
Womack, H.; French, J. C.; Johnson, F. A.; Thompson, S. S.; Swan, C. W. *Peanut Pest Management in the Southeast*; University of Georgia Cooperative Extension Service Bulletin 850; University of Georgia: Athens, GA, 1981.
Wright, D. New Method for the Determination of 1,1-Dimethylhydrazine Residues in Apples and Peaches. *J. Assoc. Off. Anal. Chem.* 1987, *70*, 718–720.

Received for review February 2, 1988. Accepted June 6, 1988.

Fluoride and Sulfate Residues in Foods Fumigated with Sulfuryl Fluoride

Rudolf H. Scheffrahn,* Ruei-Ching Hsu, Weste L. A. Osbrink, and Nan-Yao Su

Fluoride (F^-) and sulfate (SO_4^{2-}) residues in eight foodstuffs fumigated with 36 and 360 mg/L of sulfuryl fluoride (SF) for 20 h were quantified by high-performance ion chromatography. Fluoride residues were independently confirmed by F^- electrode analysis. At both SF exposures, levels of F^- and SO_4^{2-} increased concurrently within most commodity types, although in disproportionate ratios between commodities. Aeration period prior to analysis had no effect on residue levels in all commodities tested. Maximum F^- residues were observed in dried beef and maximum SO_4^{2-} residues in dry milk. Vegetable oil was virtually free of anionic residues. Neither F^- nor SO_4^{2-} residues were proportional to fumigant exposure concentrations nor to anion ratios expected from complete hydrolysis of SF.

Sulfuryl fluoride (SF) is a fumigant registered for the control of structural and household pests such as termites, wood-boring beetles, and cockroaches. For safety and convenience, the SF label specifies that food items may remain in structures during fumigation if the food is placed in air-tight containers such as 4-mil-thick (102-μm) polyethylene bags (Dow Chemical, 1982). Negligible SF residues in the ppb range have been reported from foodstuffs that were protected by polyethylene film during fumigation at 10 times the accumulated dosage (720 mg·h/L) of SF normally used for drywood termite control (Osbrink et al., 1988). Transient volatile residues of SF in unprotected foodstuffs were found to be <0.3 ppm (with the exception of vegetable oil) within 8 h after fumigation (Osbrink et al., 1988). Also, a portion of the parent compound (SO_2F_2) may be converted into fixed alteration products in certain food matrices (Meikle and Stewart, 1962). Meikle (1964) showed that graham flour fumigated for 92 h with 32 mg/L of ^{35}S-labeled SF contained nonvolatile radiolabeled alteration products. His qualitative distribution study indicated that the ^{35}S moiety was incorporated into the amino acid and protein of the flour and also resided as free sulfate (SO_4^{2-}). The fluorine constituent of degraded SF was speculated to be free fluoride (F^-), the companion product derived, in part, by phosphate-catalyzed hydrolysis of SF within the matrix. However, Meikle (1964) lacked a means for quantification and selective detection of F^-, the degradation product from SF exposure that poses a potential health risk (Dunning, 1965).

High-performance ion chromatography (HPIC) was used by Bouyoucos et al. (1983) to evaluate time-weighted exposures of humans to SF. SF collected in charcoal traps was hydrolyzed by alkaline solution, and the resultant F^- and SO_4^{2-} anions were quantified by HPIC. Concentrations of F^- in aqueous media have been successfully analyzed with electrode probes specific for detecting this anion (Liu et al., 1987; Ekstrand, 1977). In our present study, both analytical methods were adopted for verifying and quantifying the soluble anionic SF degradation products in aqueous extracts of a broad variety of fumigated food commodities.

MATERIALS AND METHODS

Note: Sulfuryl fluoride is a toxic, colorless, and odorless gas that must be handled with extreme caution by certified personnel. The TLV For SF is 5 ppm, and STEL is 10 ppm.

The following eight food items were fumigated with SF in a 4.2-m^3 chamber following the procedure of Scheffrahn et al. (1987): unbleached enriched wheat flour (Pillsbury), Kibbles 'n Bits dog food (Ken-L Ration), nonfat dry milk (Carnation), vegetable cooking oil (Crisco), dried beef, acetaminophen (Extra-Strength Tylenol, McNeilab), Red Delicious Washington apples, and Twinkies snack cakes (Hostess, individually wrapped in cellophane). The beef, dog food, and acetaminophen were finely ground in a coffee mill before fumigation to ensure homogeneity of samples.

The subdivided food samples were exposed to SF at 36 and 360 mg/L for 20 h (ca. 10 and 100 times the drywood termite rate) in open disposable cups filled with 5-g portions. The apples and snack cakes were exposed whole. After SF exposure, the samples were stored in the cups at ca. 25 °C in an air-conditioned laboratory. At 1, 8, and 15 days postfumigation, two fumigated samples and two samples of unfumigated commodity (also 5 g) from identical food lots were individually placed for 1 h on a mechanical shaker at room temperature in 50 mL of deionized water (SO_2F_2 solubility in water is 750 ppm). Prior to residue extraction, the apples and snack cake were finely chopped. Twenty milliliters of the resultant suspensions was centrifuged at 2000 rpm for 30 min. Of the supernatant formed by the centrifugation, 5 mL was passed through a C_{18} Sep-Pak column (Waters Associates), a 0.45-μm pore sized 25-mm disposable syringe filter (Cameo

Institute of Food and Agricultural Sciences, Fort Lauderdale Research and Education Center, University of Florida, 3205 College Avenue, Ft. Lauderdale, Florida 33314.

0021-8561/89/1437-0203$01.50/0