raphy (LC) for the determination of hydroxy-s-triazines avoided the derivatization of these compounds. Since the absorbance of the solvent system used for high-pressure LC is high and in order to obtain sufficient light intensity a spectrophotometer detector with an optical band width of 20 nm was required. The acidic ternary eluant system chloroform–ethanol–water resulted in the coexistence of two phases in the separation column (Hesse and Hövermann, 1973; Van den Berg et al., 1977), the polar stationary phase water retained in the pores of the silica gel support and the apolar mobile phase chloroform. A selective separation of the hydroxy-s-triazines was obtained by use of ethanol as solubility mediator. Hydroxy-s-triazines with the same molecular weight (e.g., GS 11526 and GS 23158) could not be separated with this mobile phase system. A wide range of eluant polarities could be accommodated using different amounts of ethanol. Phosphoric acid increased the solubility of hydroxy-s-triazines in the mobile phase and also increased the chromatographic resolution. It has the further advantage of being noncorrosive and transparent to UV light at 240 nm. This solvent system showed good long-term stability, although it separates in two phases at temperatures below 291 K. It could be recirculated if reciprocating pumps were used. High efficient adsorption and gel filtration columns permitted an acceptable analysis time of about 16 h/six samples and accurate quantitative determination of hydroxy-s-triazines in plant materials with limits of determination of 0.05 mg/kg.

ACKNOWLEDGMENT

The authors wish to thank A. Pfleiderer, E. Allemann, and D. Schnell for skillful technical assistance.

LITERATURE CITED

Cee, A., Gasparic, J., *J. Chromatogr.* **56**, 342 (1971).
Chen, J.-Y. T., *J. Assoc. Off. Anal. Chem.* **50**, 595 (1967).
Cochrane, W. P., *J. Chromatogr. Sci.* **13**, 246 (1975).
Fishbein, L., *Chromatogr. Rev.* **12**, 167 (1970).
Flint, G. T., Aue, W. A., *J. Chromatogr.* **52**, 478 (1970).
Hesse, C., Hövermann, W., *Chromatographia* **6**, 345 (1973).
Hurter, J., *Experientia* **22**, 741 (1966).
Jordan, L. S., Farmer, W. J., Goodin, J. R., Day, B. E., *Residue Rev.* **32**, 277 (1970).
Kaiser, R. E., *Chromatographia* **9**, 337, 463 (1976).
Karlhuber, B., Ramsteiner, K. A., CIBA-GEIGY, Internal Report: Determination of Hydroxy-Triazines, Oct 1971.
Khan, S. U., Greenhalgh, R., Cochrane, W. P., *J. Agric. Food Chem.* **23**, 430 (1975).
Khan, S. U., Marriage, P. B., *J. Agric. Food Chem.* **25**, 1408 (1977).
Kondela, S., *J. Chromatogr.* **53**, 589 (1970).
Lawrence J. F., *J. Agric. Food Chem.* **22**, 936 (1974).
Lawrence, J. F., Leduc, R., *Anal. Chem.* **50**, 1161 (1978).
Montgomery, M. L., Botsford, D. L., Freed, V. H., Meeting of the Weed Society of America, New Orleans, LA, 1968.
Ramsteiner, K. A., Hörmann, W. D., Eberle, D. O., *Meded. Rijksfac. Landbouwetensch. Gent*, **36**, 1119 (1971).
Ramsteiner, K. A., Hörmann, W. D., Eberle, D. O., *J. Assoc. Off. Anal. Chem.* **57**, 192 (1974).
Schroeder, R. S., Patel, N. R., Hedrich, L. W., Doyle, W. C., Riden, J. R., Phillips, L. V., *J. Agric. Food Chem.* **20**, 1286 (1972).
Sirons, G. J., Frank, R., Sawyer, T., *J. Agric. Food Chem.* **21**, 1016 (1973).
Van den Berg, J. H. M., Milley, J., Vonk, N., Deelder, R. S., *J. Chromatogr.* **132**, 421 (1977).

Received for review October 10, 1978. Accepted April 12, 1979. This paper was presented in part at the Fourth International Congress of Pesticide Chemistry (IUPAC), July 24–28, 1978, held in Zürich, Switzerland.

Use of High-Pressure Liquid Chromatography for Analysis of Sweet Potato Phenolics

William M. Walter, Jr.,* Albert E. Purcell, and Gary K. McCollum

A high-pressure liquid chromatographic (LC) procedure for the quantitation of sweet potato phenolics was developed. After extraction and sample cleanup, analyses were performed with a reverse-phase C-18 column using a methanol–sodium phosphate buffer system (pH 3.0). Individual phenolics were quantitated with a fixed wavelength detector operating at 313 nm. Only caffeoylquinic acid esters were identified. Chlorogenic acid and isochlorogenic acid were the most abundant components comprising 80% or more of the total. The phenolic content ranged from 14 to 51 mg/100-g sample (fresh weight) depending upon the cultivar.

Sweet potato phenolics were reported by Rudkin and Nelson (1947) to consist of chlorogenic acid and other similar compounds. Many other studies have shown that changes in the total phenolic content occurred when sweet potatoes were subjected to stress or mechanical injury. A brief discussion of this research can be found in the accompanying paper (Walter and Purcell, 1979). However, very little information is available on the identities of the phenolics or on their concentrations.

U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, and the Department of Food Science, North Carolina State University, Raleigh, North Carolina 27650.

Kojima and Uritani (1973) reported that chlorogenic and isochlorogenic acids were the major phenolics in sweet potatoes but provided no quantitative data. The only quantitative data available are those of Sondheimer (1958). He reported that in sweet potato peelings the phenolics were chlorogenic acid (3-[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5-trihydroxycyclohexanecarboxylic acid), isochlorogenic acid (-bis[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]dihydroxycyclohexanecarboxylic acid; several isomers possible), caffeic acid (3-(3,4-dihydroxyphenyl)-2-propenoic acid), neochlorogenic acid (5-[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,-3,4-trihydroxycyclohexanecarboxylic acid), and "Band 510" (4-[[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,-3,5-trihydroxycyclohexanecarboxylic acid. The levels were

335, 603, 11, 53, and 48 mg/100 g dry weight, respectively.

One reason for the lack of detailed information on sweet potato phenolics is that formerly the only means of separating such compounds was by paper and/or thin-layer chromatography which are semiquantitative or by laborious, time-consuming column chromatography. Sondheimer (1958) performed his separation on a silicic acid column by using a gradient elution procedure which required an overnight run.

The technique of high-pressure liquid chromatography (LC) offers a relatively rapid and highly selective means to separate compounds of similar structure. LC has been applied to the analysis of selected phenolic acids and flavonoids (Wulf and Nagel, 1976) and to phenolics in tobacco (Court, 1977). This study was conducted in connection with an investigation of the effects of processing on sweet potato phenolics. The purpose was to identify the phenolics in several sweet potato cultivars and to develop an LC method for quantitating them.

MATERIALS AND METHODS

Sweet potato cultivars Jewel, Centennial, Pelican Processor, Australian Canner, Julian, Porto Rico 198, and the selection NC-319 were harvested at the North Carolina Agricultural Experiment Station located in Johnston County and transported immediately to the laboratory. Three replicate samples (three roots each) were selected from each cultivar and selection. They were washed, hand-peeled, and extracted as previously described (Walter and Purcell, 1979).

Separation and Identification. Each sweet potato extract was mixed with an equal volume of 1-propanol. Sodium chloride was added with stirring until two phases separated (Bate-Smith, 1964). The lower layer was discarded and the upper layer removed and evaporated in vacuo. The residue was extracted with methanol. The methanolic extract was applied as a streak onto sheets of Whatman No. 3 chromatography paper and developed overnight (descending) using a butanol–acetic acid–water (4:1:2) solvent system. The sheets were air-dried and examined under ultraviolet light. Portions of the chromatograms containing fluorescent bands were extracted with 80% ethanol. Each band extract was concentrated and streaked onto thin-layer chromatography (TLC) plates coated with (100 μm) microcrystalline cellulose (Applied Science Laboratory) which had been purified by sequential extraction with ethanol, methanol, and ether before plate preparation. The TLC procedure which served as the final purification step was carried out using formic acid–water (2:98) as the solvent. Purified bands were removed from the adsorbent with 80% ethanol.

For the purpose of identification, portions of the purified compounds were placed on sheets of Whatman No. 1 chromatography paper and developed in four solvent systems: (1) BAW, 1-butanol–acetic acid–water (4:1:2); (2) BPW, 1-butanol–pyridine–water (14:3:3); (3) HOAc, glacial acetic acid–water (2:98); (4) BzAW, benzene–acetic acid–water (125:72:3).

Using the BAW system, chromatograms were prepared such that each purified compound could be examined in uv light before and after fuming with NH_3 and tested with $FeCl_3$–$K_3Fe(CN)_6$ reagent (Ribereau-Gayon, 1972), diazotized *p*-nitroaniline, DPNA (Ribereau-Gayon, 1972), and the Hoepfner reagent (Schaller and Von Elbe, 1970). In addition, each purified compound was diluted, as required, with methanol and the spectra before and after addition of aqueous sodium hydroxide were recorded from 220 to 460 nm. Authentic samples of chlorogenic and caffeic acids (Sigma Chemical Co.) and isochlorogenic acid (K and K Laboratories) were carried along with the purified samples through chromatography, spectrophotometry, and hydrolysis.

Hydrolysis. Each TLC purified compound (~0.1 mg) was mixed with 5 mL of 2 N HCl and refluxed at 100 °C for 1 h. The hydrolysate was extracted three times with ethyl ether, and the combined extracts were dried and concentrated. Concentrates were chromatographed with the solvent systems 1–4. The aqueous phase was evaporated to dryness in vacuo and a portion chromatographed (Whatman No. 1 paper) along with a standard mixture of glucose, xylose, arabinose, and rhamnose. Butanol–benzene–pyridine–water (5:1:3:3) was the solvent system. After development and drying, the chromatogram was sprayed with a solution containing 0.45 g of oxalic acid and 0.9 g of aniline acid in 100 mL of water. Heating the chromatogram at 105 °C for 15 min resulted in the appearance of various colored spots depending upon the sugar.

A second portion of the aqueous phase of the hydrolysate was chromatographed on Whatman No. 1 paper. Quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid; Sigma Chemical Co.) was run along with the hydrolysate. The chromatogram was developed in the solvent system butanol–formic acid–water (4:1:2) and sprayed with sodium metaperiodate and sodium nitroprusside–piperazine according to Cartwright and Roberts (1955). Quinic acid appeared as a bright yellow spot.

High-Pressure Liquid Chromatography. *Preanalysis Cleanup.* This step was necessary to prevent progressive deterioration of column resolution, probably due to irreversible adsorption of nonphenolic material. Cleanup consisted of passing each sample through the same type of reverse-phase materials as that which was to be used in the analytical column.

An aliquot of each sweet potato extract (Walter and Purcell, 1979) was diluted with water so that the total phenolic level was ~0.2 mg/mL. Then, 1 mL of the sample was mixed with 1.0 mL of internal standard. The combined solution was added to a 12.9 × 0.7 cm pipet plugged with glass wool and containing 0.2 g of Bondapak C_{18}/Porasil B (Waters Associates) which had been washed with 2 mL of acetonitrile followed by 2 mL of water. The sample was allowed to pass through the column under slight nitrogen pressure. The column was then treated with 5 mL of acetonitrile–methanol–water (40:40:20) to remove the adsorbed phenolics and the eluate analyzed by LC.

Identification and Quantitation. The instrument used was a Waters Associates ALC/GPC-204 LC equipped with a U6K injector, a precolumn containing Co:Pell ODS (Whatman Associates) and a 3.9 mm i.d. × 300 mm μBondapak C_{18} column (Waters Associates). The solvent (40% methanol–60% 0.033 M phosphate buffer, pH 3.0) was delivered isocratically at a flow rate of 0.8 mL/min. The column effluent was monitored at 313 nm (0.02 aufs), and the response recorded on a strip-chart recorder. Samples of the phenolic compounds purified by PC and TLC were chromatographed to establish their elution times.

The internal standard method of quantitation was used to compensate for errors in the analytical procedure (Eng et al., 1977). To standardize the procedure, a series of solutions containing 20–125 μg/mL of chlorogenic acid (CA) and 100 μg/mL of coumarin (internal standard) were injected, and the peak areas were measured with a planimeter. From these data, the internal standard constant, K_i, was calculated and found to vary 3% over

Table I. R_f Values and Adsorption Maxima of Phenolic Compounds in Sweet Potato

compd	R_f[a] BAW	BPW	HOAC	B_2AW	color in uv normal	NH_3	spectrum in 80% EtOH normal	with NaOH	tentative identity
1	0.80	0.42	0.12	0.49	blue	green	323	343	isochlorogenic acid (ICA)
2	0.71	0.13	0.54	0.40	blue	green	323	340	chlorogenic acid (CA)
3	0.56	0.40	0.71	0.57	blue	blue	323	340	4-O-caffeoylquinic acid
4	0.62	0.40	0.70	0.58	purple	purple			
5[b]	0.48	0.32	0.59	0.26	blue	green	323	342	neochlorogenic acid
CA	0.69	0.14	0.56	0.38	blue	green			
CAF[c]	0.81	0.49	0.27	0.56	blue	blue			
ICA	0.78	0.44	0.11	0.48	blue	green			

[a] Whatman No. 1 chromatography paper in solvent systems described in manuscript. [b] This compound present in cv. Jewel stored >4 weeks. [c] Caffeic acid.

Table II. Color Responses of Spray Reagents toward Phenolic Compounds from Sweet Potato

compd	color response $K_3Fe(CN)_6$	DPNA	Hoepfner normal	Hoepfner with base
1	blue	tan	brown	brown-red
2	blue	tan	brown	red
3	blue	brown		
4	blue	orange		
5	blue	tan	brown	brown-red

the concentration range studied. The low variation thus indicated that CA solutions obeyed Beer's law. Each sample was then chromatographed (3–7 μL) in duplicate, and the peak areas were measured. The peak areas were quantitated as CA by using K_i, the ratio of the coumarin area to sample peak area, and the appropriate dilution factors.

Statistical Analysis. The effect of cultivar or selection upon the concentrations of the individual phenolic compounds was determined by analysis of variance and the Waller-Duncan K-ratio T test (SAS, 1976).

RESULTS AND DISCUSSION

Isolation and Identification. Five compounds were isolated by preparatory PC of the extract from cv. Jewel. All were colorless in visible light, but were fluorescent in both short- and long-wavelength UV. Compounds 1, 2, and 5 (Table I) appeared blue under UV illumination, changing to green when fumed with NH_3 vapor. This is characteristic of esters of caffeic acid. Compounds 3 and 4 did not appear to change color in NH_3 vapor. However, these compounds were present in very small amounts, and color changes would have been difficult to observe. The UV spectra of all the isolated compounds except 4 had a λ max in the area of 322–326 nm which shifted to 340 nm upon the addition of sodium hydroxide. These spectral characteristics are also shown by caffeic acid esters. Compound 4 had no maximum in the UV. Compounds 1, 2, and 5 responded similarly to spray reagents (Table II): (1) blue with $K_3Fe(CN)_6$, (2) tan with DPNA, (3) brown changing to brown-red with base after nitrous acid spray (Hoepfner Reagent). These responses are to be expected from caffeoyl esters. Compounds 3 and 4 gave faint but positive responses to $K_3Fe(CN)_6$ and to DPNA but no response to the Hoepfner test.

The R_f of the various compounds in four solvent systems indicated that 1 was similar to isochlorogenic acid (ICA) and that compound 2 was similar to CA. Caffeic acid (CAF), the other standard, did not appear to be present.

Each of the compounds was acid hydrolyzed and separated into its aglycon and water-soluble residue. Paper chromatography of the aglycon with BAW and 2% HOAc revealed that compounds 1, 2, 3, and 5 were CAF derivatives. Compound 4 did not appear to contain CAF. With the exception of 4, all appeared to contain glucose. In addition, hydrolysates from 1, 2, and 5 gave chromatograms with R_f and color reactions the same as those of the quinic acid standard. Compound 3 gave a very weak response presumably due to the small amount of material available for hydrolysis. Compound 4 gave no evidence of containing quinic acid.

Since Jewel was readily available, this cultivar was used for all isolation and identification procedures. Chromatographic examination of the extracts from the other cultivars and from freshly harvested Jewel showed that they did not contain compound 5. Subsequently, additional tests with Jewel showed that this compound (5) was not present at harvest, but became detectable about 4 weeks after harvest. Otherwise, the same compounds were present in all cultivars. The evidence from the analysis of standards and from the hydrolysates is that compound 1 was ICA, compound 2 was CA, compound 3 was probably 4-*O*-caffeoylquinic acid, compound 4 was probably not a phenolic, and compound 5 was a CA isomer possibly neochlorogenic acid (Scarpati and Esposito, 1964). The identities of compounds 3 and 5 were assigned on the basis of the order of elution in a LC separation reported by Court (1977). With the exception of compound 4, all of the phenolics appeared to be glucosides. However, it should be noted that molar ratios of glucose to phenolics were not obtained. It is possible that glucose found in the acid hydrolysates is an artifact. Thus its participation in glycosidic linkages must be considered tentative.

Quantitation by LC. Test studies with the cleanup column indicated that about 10% of the UV-absorbing material was not retained by the column. The phenolics were retained initially but were completely removed by methanol–acetonitrile–water. Examination of the spectrum of the nonretained material indicated a broad, nonspecific absorbance with no maximum in the region of 323 nm. Thus this material was probably not phenolic.

After cleanup, the phenol-containing elute was resolved into its components by LC. In preliminary studies with a series of solvent systems containing water, methanol, and acetic acid (Wulf and Nagel, 1976), unacceptable resolution was obtained. Substitution of acetonitrile for methanol did not improve resolution. However, when the methanol–phosphate system of Court (1977) was used, resolution significantly improved. Separations were best when the column was isocratically eluted with a solution of 40 mL of methanol, 60 mL of 0.033 M phosphate, and 0.4 mL of glacial acetic acid (pH 3.0) to prevent tailing. Attempts to use the same system, but without phosphate, were unsuccessful. Apparently, the phosphate modified the adsorption characteristics of the C_{18} packing sufficiently

Table III. Phenolic Composition[a,b] of Sweet Potato Cultivars as Determined by LC

cultivar	chlorogenic acid	4-O-caffeoylquinic acid[c]	isochlorogenic acid-1	isochlorogenic acid-2	total phenolics
Jewel	9.98[a] (53.93)[d]		0.92[a] (4.97)	7.61[a] (41.12)	18.51[a]
Centennial	7.92[b] (55.86)		0.74[a] (5.22)	5.52[b] (38.93)	14.18[b]
Julian	11.00[c] (49.16)		1.77[b] (7.64)	9.67[c] (43.22)	22.38[c]
NC-319	11.07[c] (45.10)		2.19[c] (8.92)	11.28[d] (45.96)	24.54[d]
Pelican Processor	12.74[d] (31.96)	2.56[a] (8.11)	1.66[b] (5.27)	14.60[e] (46.27)	31.56[e]
Porto Rico 198	14.84[e] (43.91)	1.29[b] (3.82)	4.29[e] (12.69)	13.37[f] (39.56)	33.80[f]
Australian Canner	20.27[f] (39.56)	3.66[c] (7.14)	4.10[e] (8.00)	23.21[f] (45.30)	51.24[g]

[a] Milligram in 100 g fresh weight; results calculated from duplicate analyses of three replicates. [b] Numbers with the same letter in a vertical column are not significantly different ($P < 0.05$). [c] Tentative identification. [d] Numbers in parentheses are percent composition within cultivars.

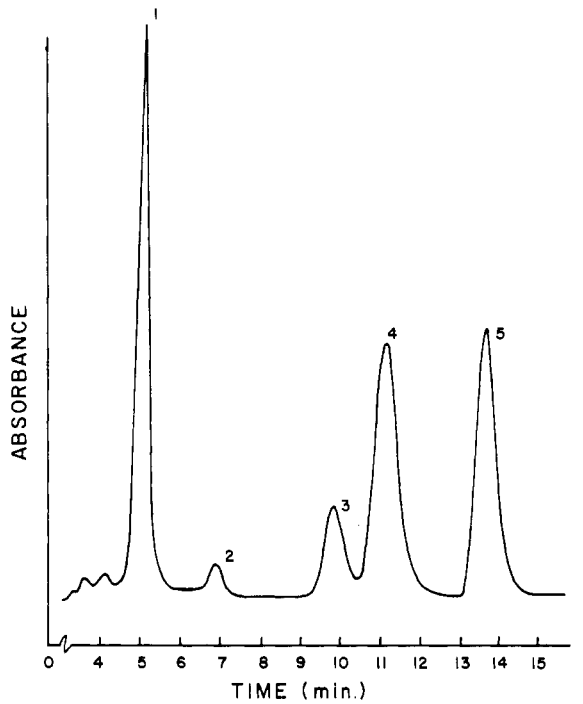

Figure 1. LC of phenolics from Pelican Processor. Compounds: (1) chlorogenic acid, (2) 4-O-caffeoylquinic acid (tentative identification), (3) isochlorogenic acid-1, (4) isochlorogenic acid-2, (5) coumarin (internal standard).

to allow good separation. In this system, compounds 2 and 5 were coeluted. For adequate separation either a gradient must be employed or each sample must be eluted with two solvent systems. Since compound 5 was not present in freshly harvested roots, the system used was adequate.

Figure 1 shows the separation obtained on the μBondapak C_{18} column. Chlorogenic acid was eluted first and was followed by 4-O-caffeoylquinic acid. The next two peaks corresponded to the isomeric isochlorogenic acids. Scarpatti and Esposito (1964) reported that ICA includes three dicaffeoylquinic acid isomers. The higher molecular weights involved explain why these compounds elute late in the analysis. It is not known which of the isomers corresponds to which peak. The procedure did not provide separation of all three isomers. Using authentic ICA, an elution pattern identical with that of sweet potato ICA was obtained. The compound designated as ICA-2 was the major isomer.

The LC procedure eliminates the need to remove phenolics from aqueous solution by extraction with a hydrophobic organic solvent (e.g., ethyl acetate) prior to application onto the chromatographic medium. Studies have shown that solvent extraction with ethyl acetate, which is required prior to PC or TLC, left considerable amounts of phenolic material in the aqueous phase. Although the LC method described herein involves a preanalysis cleanup, essentially quantitative recovery of phenolics was attained.

The analytical results (Table III) indicated that CA and ICA-2 were the most abundant phenolics. Chlorogenic acid was the most abundant in four of the cultivars and ICA was the most abundant in NC-319, Pelican Processor, and Australian Canner. The phenolic levels ranged from 14.18 to 51.24 mg/100 g of fresh tissue. Samples were obtained only for 1 year and at one location; consequently, the data on phenolic levels are subject to confirmation. Nevertheless, the LC method does appear to provide an adequate means of identifying and quantitating sweet potato phenolics.

LITERATURE CITED

Bate-Smith, E. C., "Methods in Polyphenol Chemistry", Pridham, J. B., Ed., Pergammon Press, Oxford, England, 1964.
Cartwright, R. A., Roberts, E. A. H., *Chem. Ind.*, 230 (1955).
Court, W. A., *J. Chromatogr.* **130**, 287 (1977).
Eng, M.-Y., Calayan, C., Talmage, J. M., *J. Food Sci.* **42**, 1060 (1977).
Kojima, M., Uritani, I., *Plant Physiol.* **51**, 768 (1973).
Ribereau-Gayon, P., "Plant Phenolics", Hafner Publishing Co., New York, 1972, pp 67–68.
Rudkin, G. O., Nelson, J. M., *J. Am. Chem. Soc.* **69**, 1470 (1947).
SAS, "A Users Guide to SAS", Bair, A. J., Goodnight, J. H., Sall, J. P., Helwig, J. T., SAS Institute, Raleigh, NC, 1976, pp 108–109.
Scarpati, M. L., Esposito, P., *Ann. Chim. (Rome)* **54**, 35 (1964).
Schaller, D. R., Von Elbe, J. H., *J. Food Sci.* **35**, 762 (1970).
Sondheimer, E., *Arch. Biochem. Biophys.* **74**, 131 (1958).
Walter, W. M., Jr., Purcell, A. E., *J. Agric. Food Chem.*, preceding paper in this issue (1979).
Wulf, L. W., Nagel, C. W., *J. Chromatogr.* **116**, 271 (1976).

Received for review January 22, 1979. Accepted April 25, 1979. Presented at the 176th National Meeting of the American Chemical Society, Miami Beach, FL, Sept 1978. Paper No. 5879 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina State University, nor does it imply approval to exclusion of other products that may be suitable.