Table I. Influence of Boiling Time and pH in the Isolation Step on the Recovery of C-T from MDPM, Spiked with 1 ppm of C-T

boiling time, min	pH	% recovery
10	6.9	45
20	6.9	60
30	6.9	68
20	6.9	60
20	5.9	64
20	4.9	76
20	4.5	gel formation
30	4.9	84

Table II. Recoveries of C-T from Different Foodstuffs, Spiked with 1 ppm of C-T

product	no. of analysis	av % recov. (P = 95%)	SD, %
dist. water	7	89.4 ± 3.7	4.0
milk	10	86.4 ± 6.3	8.8
ice cream	8	91.3 ± 7.5	9.0
MDPM	10	82.6 ± 4.2	5.9
whole egg	10	69.6 ± 2.4	3.4
croquette	9	77.6 ± 2.2	2.9

deviations were determined to check repeatability (reproducibility). Table II shows the results for MDPM and the other products for seven–ten samples of each product after spiking with 1 ppm of C-T.

The proposed method is suitable for the determination of C-T at a desired level in a wide range of products and can be performed with a readily controllable system (GC/FID), which is suitable for routine analysis. The recovery and reproducibility (Table II) of this method are in good agreement with the results of other authors (Van der Haar and Veenkamp, 1977; Rondags and Beljaars, 1978) but it is more sensitive and applicable to a variety of foods.

Finally two remarks: although system saturation can be performed easily, it takes at least 1 h. As an alternative, a glass column cannot be used because of the poor heat transfer that would make temperature programming ineffective. Possibly the new nickel tubing would help this problem.

In other laboratories another problem can be temperature programming with 32 °C/min. Most modern gas chromatographs can easily maintain this rapid rise. For older types which cannot it will be necessary to try a rate higher than 16 °C/min, for this rate does not yield a proper separation of p-TS from interfering peaks. In an early stage of our work a sample cleanup was tried (the EtAc extracts were washed with a 0.5 M solution of sodium hydroxide) but this led to lower recoveries (Steverink, 1977). Possibly another type of sample cleanup will do the job (e.g., column chromatography).

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High-Pressure Liquid Chromatographic Determination of Hydroxy-*s*-triazine Residues in Plant Material

K. A. Ramsteiner and W. D. Hörmann*

A specific method is described for quantitative determination of hydroxy-*s*-triazine residues in biological material. The hydroxy metabolites of atrazine, simazine, propazine, terbutylazine, and the corresponding methylthio and methoxy compounds are separated by high-pressure liquid chromatography on a silica gel column and detected at 240 nm with a UV spectrophotometer detector. The procedure involves the extraction of samples with methanol, cleanup with a strong cation-exchange resin, a polyacrylamide adsorption resin, and a styrenedivinylbenzene gel filtration column. The clean-up procedure described is not suitable for dealkylated hydroxy-*s*-triazine metabolites; however, those compounds are separated chromatographically also under the liquid chromatographic conditions given. Recoveries in the range of 70–113% indicated that this procedure is suitable to the residue analysis of hydroxy-*s*-triazines without derivatization of the metabolites with detection limits of 0.05 mg/kg.

An important pathway for the degradation of *s*-triazine herbicides in soil and other biotopes is their conversion to the corresponding hydroxy analogues (Jordan et al., 1970). These represent major alteration products after hydrolytic cleavage of the groups located in position 6. Hydroxy-*s*-triazines exist as tautomeric mixtures involving ring protonated structures (Chen, 1967; Jordan et al., 1970).

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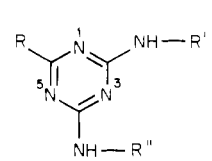

R = Cl, OCH_3, SCH_3

The chemistry of the hydroxy-*s*-triazines is not similar to that of alcohols, phenols, or amides, and due to their physicochemical properties (low volatility, melting points above 580 K, low solubility in organic solvents, chemical

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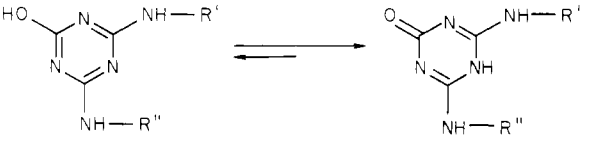


inertness, see also Table I), none of the residue analytical methods reported so far were found to be suitable for routine work.

Currently, hydroxy-s-triazines are determined by TLC (Kondela, 1970; Cee and Gasparic, 1971) or UV spectrophotometry (Hurter, 1966; Sirons et al., 1973).

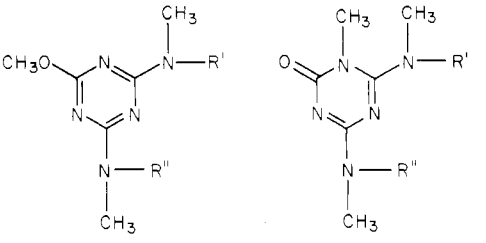
While gas–liquid chromatography with various specific detectors was employed successfully for residue analysis of parent triazines by Fishbein (1970) and Ramsteiner et al. (1971, 1974), hydroxy-s-triazine compounds were not directly amenable to this very specific and sensitive technique. Some reports, which we found in literature, were concerned with the formation of volatile derivatives to allow gas chromatographic determination of hydroxy-s-triazines. Silylation procedures were described first for the derivative formation of hydroxy-s-triazine compounds by Montgomery et al. (1968), Flint and Aue (1970), and Khan et al. (1975). Reaction conditions should be carefully observed, since the reaction can lead to mono-, di-, and trisilyl derivatives. Silylation reactions are not recommended for use with alkali flame ionization detectors and most other, commonly used, specific detectors for residue determination. Poisoning of the salt tip or catalysts and, therefore, loss of sensitivity will result after repeated injections. These limitations disqualify the silylation procedures for most residue analytical problems.

Schroeder et al. (1972) described a procedure which was based on conversion of the hydroxy-s-triazine of cyprazine (2-chloro-4-cyclopropylamino-6-isopropylamino-s-triazine) back to the original chloro-s-triazine and its subsequent determination by gas chromatography. Interfering plant background was separated from the hydroxy-s-triazine compound by two types of chromatographic columns. Recoveries in corn samples (grain and silage) were reported to be in the range of 62–88% at fortification levels of 0.1–0.2 mg/kg. Khan et al. (1975) and Khan and Marriage (1977) described a methylation procedure for hydroxyatrazine with diazomethane. Hydroxyatrazine was converted to atraton with 50–80% yields. These results were confirmed by our own experiments. Considerable variation in the amount of derivatized hydroxyatrazine was obtained and the reaction can be denoted to be only of qualitative value.

Alkylation with methyl iodide in strongly basic solutions was used for confirmation of triazine residues by Lawrence (1974) and Cochrane (1975). The same reaction was also successfully used for derivatization of hydroxyatrazine (Khan et al., 1975) with average yields of the trimethylated derivative of 60–80%. Recoveries of fortified soil samples after derivatization by the alkylation method ranged from 53 to 60%. These results agreed with the recoveries found in this laboratory by Karlhuber and Ramsteiner (1971) with a slightly different alkylation procedure.

Khan et al. (1975) postulated an N,N′-dimethylatraton (I) based on MS and NMR spectra, whereas, in this laboratory, evidence for ring nitrogen methylation (II) was found by MS and IR studies.

The goal of the work presented in this paper was to develop a straightforward, specific, quantitative, and reproducible method for the determination of residues of hydroxy-s-triazines in plant material. If possible, the different hydroxy-s-triazines (Table I) should be separated from each other. High-pressure liquid chromatography



I II

was the method chosen according to the physicochemical properties of these compounds. A first approach for the separation of hydroxy-s-triazines was recently published by Lawrence and Leduc (1978). Six hydroxy-s-triazines were separated on 5 μm of silica gel with a mobile phase consisting of 10% methanol in dichloromethane containing 0.1 mol/L of propionic acid.

EXPERIMENTAL SECTION

Sample Preparation. A representative 800–1000-g subsample of wet plant leaf material was chopped in the food cutter. A 45-g subsample was placed in a 500-cm^3, wide-mouthed jar. Methanol (200 cm^3) was added and the plant material was macerated with the high-speed homogenizer. A 500-cm^3 round-bottommed flask was attached to the extractor (Figure 1). The slurry was transferred to the glass insert of the hot extractor. The jar was rinsed twice with 50 cm^3 of methanol, and the rinsings were also transferred to the insert. The sample was extracted for 4 h.

Dry plant materials (straw or grains) were entirely ground together with dry ice in a cross-beater mill. After evaporation of the dry ice, a subsample of 45 g (15 g only of straw samples) was placed in the glass insert of the hot extractor. Methanol (300 cm^3) was used for a 4-h extraction period under reflux.

Cleanup. *Cation-Exchange Column.* To prevent extraction of interfering material from the resin, a pretreatment was necessary. Dowex 50 W-X4, particle size 150–300 μm, was refluxed for a 10-min period with a 1:1 mixture of 25% ammonia and deionized water. The resin was washed neutral with deionized water and then refluxed for a further 10-min period with a 1:1 mixture of 37% hydrochloric acid and deionized water. The resin was rinsed until the washings became neutral. It was now ready for column chromatography. Pretreated Dowex 50 W-X4 resin, (30 cm^3) was filled in a chromatographic tube of 23 mm i.d. and 30 cm length. The entire methanolic plant extract was transferred to the column. The flask was rinsed successively with 100 cm^3 of 50% aqueous methanol, 100 cm^3 of 1 M hydrochloric acid, and 100 cm^3 of water. All washings were one after the other and transferred to the column, and the eluates were discarded. The hydroxy-s-triazines were eluted from the column with 350 cm^3 of 5% ammonia. This eluate was collected and evaporated to dryness.

Adsorption Column. A methanolic suspension of 30 cm^3 Amberlite XAD-2, a polyacrylamide resin, particle size 100–200 μm (Rohm and Haas) was filled into a chromatographic tube of 23 mm i.d. and 30 cm length to yield a column bed of 70 ± 5 mm length. The freshly prepared column was subsequently rinsed with 100 cm^3 of deionized water and 100 cm^3 of 0.1 M hydrochloric acid. The residue of the eluate from the cation-exchange column was dissolved in 100 cm^3 of 0.1 M hydrochloric acid by swirling the flask in an ultrasonic bath for about 2 min. The solution was transferred to the column. The column was washed with 100 cm^3 of deionized water and 10 cm^3 of methanol. The hydroxy-s-triazines were eluted with 25 cm^3

Table I. Chemical Names, Parent Herbicides, and Physical Properties of Hydroxy-*s*-triazines

code no.	chemical name	parent herbicide	mol wt	solubil. in water
G 30414	2,4-bis(ethylamino)-6-hydroxy-*s*-triazine	simazine simeton simetryn	183.2	6
G 34048	2-(ethylamino)-6-hydroxy-4-(isopropylamino)-*s*-trizine	atrazine atraton ametryn	197.2	16
GS 11526	2,4-bis(isopropylamino)-6-hydroxy-*s*-triazine	propazine prometon prometryn	211.3	55
GS 23158	2-(*tert*-butylamino)-4-(ethylamino)-6-hydroxy-*s*-triazine	terbutylazine terbutryn terbumeton	211.3	4.5
GS 28784	4-(*sec*-butylamino)-2-(ethylamino)-6-hydroxy-*s*-triazine	secbumeton	211.3	
GS 17792	2-amino-4-(ethylamino)-6-hydroxy-*s*-triazine		155.5	79
GS 17794	2-amino-6-hydroxy-4-(isopropylamino)-*s*-triazine		169.2	58
GS 28620	2-amino-4-(*tert*-butylamino)-6-hydroxy-*s*-triazine		183.2	17
GS 28785	2-amino-4-(*sec*-butylamino)-6-hydroxy-*s*-triazine		183.2	62

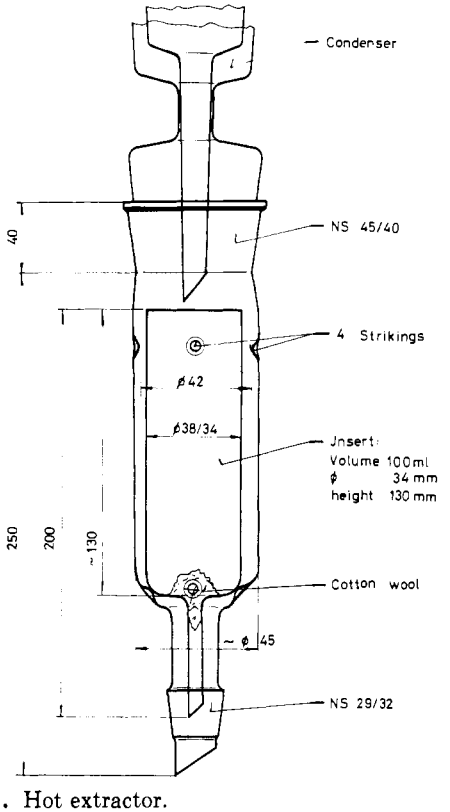


Figure 1. Hot extractor.

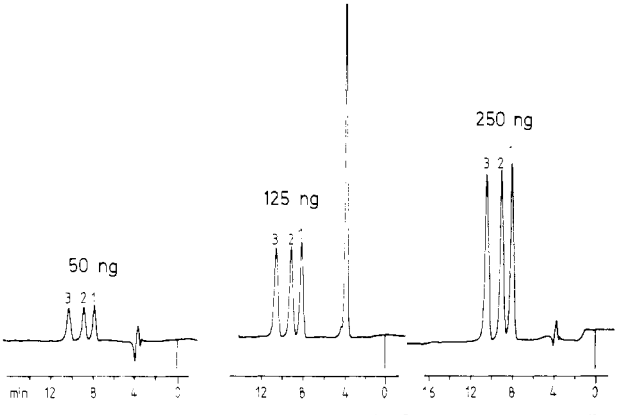


Figure 2. Chromatograms of parent hydroxy-*s*-triazines: flow rate, 0.5 cm^3/min; sensitivity, 0.1 absorbance unit full scale, 240 nm; (1) GS 11526, (2) G 34048, and (3) G 30414.

of methanol. This eluate was collected and evaporated to dryness.

Gel Filtration Column. An adjustable precision column of 10 mm i.d. × 60 cm length, capable of operating up to 100 kPa (e.g., Chromaflex, Kontes Glass Co., NJ) was assembled according to the instructions from the manufacturer.

Bio-Gel P-2 (Bio-Rad Laboratories), a styrenedivinylbenzene copolymer resin, was soaked for 4 h in 0.01 M hydrochloric acid. The column was filled to 50-cm bed height. While pumping solvent at a rate of 1 cm^3/min through the column by a reciprocating membrane piston pump, a standard solution of hydroxy-*s*-triazines was injected. Fractions of 1 cm^3 were collected and checked for hydroxy-*s*-triazines content. Normally, for a 50-cm column, more than 95% of the hydroxy-*s*-triazines eluted in the 30–45-cm^3 fraction.

The residue of the eluate from the adsorption column was dissolved in 1.5 cm^3 of 0.01 M hydrochloric acid. One cubic centimeter of this solution was injected onto the column. The 15-cm^3 fraction containing the hydroxy-*s*-triazines was collected in a graduated test tube and evaporated to dryness.

High-Pressure Liquid Chromatography. A stainless steel column of 3 mm i.d., filled with LiChrospher, SI 60 (E. Merck AG, Darmstadt, FRG) with an average particle size of 10 μm, was used. This column was attached to an ORLITA SK 15-3 (Orlita KG, Giessen, FRG) reciprocating membrane pump and a spectrophotometer model UVIKON LCD 725 (Kontron AG, Zürich, Switzerland). To achieve separation of hydroxy-*s*-triazines, a column performance of about 6000 real plates (N_{real}, $k' = 10$) according to Kaiser (1976), was necessary. The column was operated at ambient temperature with a flow rate of 0.5 cm^3/min, resulting in a pressure of about 10 MPa. The mobile system consisted of a mixture of 700 cm^3 of chloroform, 300 cm^3 of methanol, 60 cm^3 of water, and 1 cm^3 of 87% phosphoric acid. All solvents were of analytical grade. The chloroform was dried over anhydrous sodium sulfate before use in order to ensure constant water content. The detector was operated at 240-nm wavelength with an optical band width of 20 nm and 0.1 absorbance unit full-scale recorder deflection. Standard solutions of 1 μg/cm^3 up to 10 μg/cm^3 mobile phase were prepared. Fifty cubic millimeters of four different standard solutions were dosed. A linear plot of the hydroxy-*s*-triazine concentrations vs. the recorder responses (peak heights) yielded a straight line. The dry residue of the eluate from the gel filtration column was dissolved in an appropriate volume of the mobile phase so that with a 50-mm^3 sample

Table II. Retention Times of Hydroxy-s-triazines

dialkylhydroxy-s-triazines: flow rate, 0.5 cm³/min (1 cm³/min)		
G 30414	630 s	(480 s)
G 34048	550 s	(400 s)
GS 11526	480 s	(340 s)
GS 23158	480 s	(340 s)
GS 28784	480 s	(340 s)
monodealkylhydroxy-s-triazines: flow rate, 1 cm³/min		
GS 17792	995 s	
GS 17794	745 s	
GS 28620	595 s	
GS 28785	595 s	

Table III. Typical Recoveries of Hydroxy-s-triazines

sample	fortification level, mg/kg	percent recovery G 11526	G 34048	GS 30414
wheat grain	0.1	113	92	110
	0.5	95	92	98
wheat straw	0.2	93	85	81
	0.5	84	87	92
maize grain	0.1	91	92	105
	0.5	85	90	92
maize stalks	0.1	94	77	79
	0.5	83	81	76
silage maize	0.1	81	83	91
	0.5	73	84	80

loop a maximum of a 500-mg sample aliquot is dosed.

Representative chromatograms are shown in Figures 2, 3, and 4. Retention times of hydroxy-s-triazines are shown in Table II.

RESULTS AND DISCUSSION

The average recoveries of G 30414, G 34048, and GS 11526 at two levels of fortification in different plant parts are given in Table III.

Studies to pinpoint the most critical clean-up step revealed that the extraction caused the highest losses of the hydroxy-s-triazine added. In order to check the extraction procedure, experiments with different solvent systems and extraction procedures were performed. Comparative trials between the described 4-h exhaustive hot extraction, refluxing for 2 h, or mechanical shaking of silage maize samples, fortified just before extraction, are shown in Table IV.

Acidic or basic solvent systems reduced the overall recoveries compared with straight methanol shaking. Based on these experiments and recovery studies with other plant parts, the hot extraction procedure was chosen.

The clean-up procedure described could not be used for the determination of dealkylated hydroxy-s-triazines.

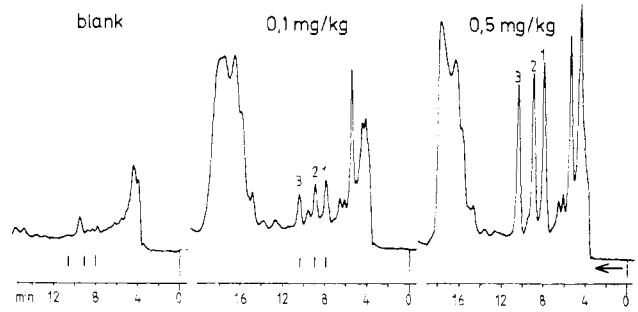


Figure 3. Chromatograms of wheat grain spiked with parent hydroxy-s-triazines. Conditions same as in Figure 2. Equivalent of 500-mg sample injected.

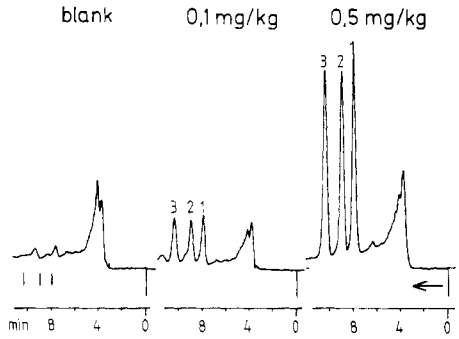


Figure 4. Chromatograms of maize grain spiked with hydroxy-s-triazines. Conditions same as in Figure 2. Equivalent of 500-mg sample injected.

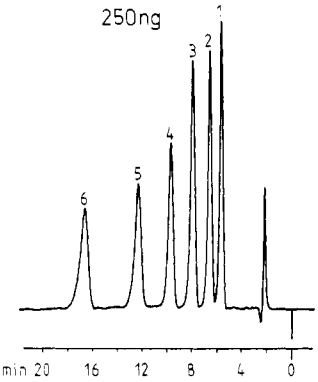


Figure 5. Separation of hydroxy-s-triazines: flow rate, 1 cm³/min; sensitivity, 0.1 absorbance unit full scale, 235 nm; (1) GS 11 526, (2) G 34 048, (3) G 30 414, (4) GS 28 620, (5) GS 17 794, and (6) GS 17 792.

These compounds were irreversibly adsorbed on the XAD-2 resin. They can, however, be separated under the high-pressure liquid chromatographic conditions given (Figure 5). The use of high-pressure liquid chromatog-

Table IV. Recoveries from Silage Maize Fortified with 0.5 mg/kg Using Different Extraction Procedures

extraction procedure	solvent system	% recoveries GS 11526	G 34048	G 30414
4-h hot Soxhlet extraction	methanol	80	84	73
2-h reflux	methanol			66
2-h mechanical shaking after blending	methanol	77	71	60
2-h mechanical shaking after blending	180 cm³ of methanol, 20 cm³ of 0.1 N HCl	53	63	53
2-h mechanical shaking after blending	180 cm³ of methanol, 20 cm³ of 1 N HCl	50	66	50
2-h mechanical shaking after blending	200 cm³ of methanol, 10 cm³ of HCl concn.	22	28	37
2-h mechanical shaking after blending	165 cm³ of methanol, 35 cm³ of TMAH[a]	<15	<15	<15
2-h mechanical shaking after blending	193.5 cm³ of methanol, 7.5 cm³ of TMAH	66	64	62
2-h mechanical shaking after blending	199 cm³ of methanol, 0.75 cm³ of TMAH	82	76	66

[a] TMAH = tetramethylammonium hydroxide solution 25% in methanol (all extracts were acidified before ion-exchange column cleanup).

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

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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.

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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