Distribution of Phenols in "Jewel" Sweet Potato [*Ipomoea batatas* (L.) Lam.] Roots

William M. Walter, Jr.,* and William E. Schadel

"Jewel" cultivar sweet potato roots were dissected into cross sections representing proximal (stem), middle (midroot), and distal (root) segments. Tissue from each segment was divided into skin, secondary tissue external to the cambium (outer; ~5-mm diameter), and secondary tissue internal to the cambium (inner). The quantity of phenolics for these tissue segments was measured by using a single-pump liquid chromatograph equipped with an inexpensive gradient-forming device. Phenolics decreased in the order outer > skin > inner. The phenol levels of the inner tissue were uniform throughout the root, while the outer tissue of the stem and root ends were found to contain more phenolics than the midroot outer tissue. Thus, ~78% of the phenolics were found to be localized in the skin and outer 5 mm of tissue.

Darkening or greying of canned sweet potatoes after exposure to air has been attributed to the interaction between polyphenol oxidase (PPO; *o*-diphenol:O_2 oxidoreductase, EC 1.10.3.1) and phenolics caused by tissue injury during the lye-peeling step of the canning process (Scott et al., 1944; Scott and Kattan, 1957). Walter and Purcell (1980) were able to show that the amount of darkening in homogenized sweet potato was directly proportional to the concentration of phenolics and that the majority of the phenolics were esters formed between quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid) and the *o*-dihydroxyphenol caffeic acid [3-(3,4-dihydroxyphenyl)-2-propenoic acid]. These are the chlorogenic acids. Hoover (1963) controlled darkening in lye-peeled roots used to prepare dehydrated sweet potato flakes by adding a mixture of phosphate salts to the cooked roots immediately prior to comminution. He postulated that the darkening was due to the oxidation of *o*-dihydroxyphenol (DHP) complexed iron. The added phosphates bound iron more strongly than did the phenolics and, thus, displaced the DHP from the complex. The DHP–iron complex has also been implicated in the after-cooking discoloration of white potatoes (Hughes and Swain, 1962).

In view of the importance of DHP to color of processed sweet potato products, it is essential to know the distribution, quantity, and identity of these compounds in the sweet potato root. Qualitative histochemical tests have shown the anatomical localization of DHP to be in the phellem, phellogen, and phelloderm, in ~1 mm (~10–15 cells) of the tissue directly beneath the periderm, in the latex of laticifers (~3–5 mm beneath the skin), in the phloem, in the cambium, which separates the secondary phloem from the secondary xylem, in the anomalous secondary cambia of the central core, in the parenchyma cells adjacent to the xylem elements, and in the walls of the xylem elements (Schadel and Walter, 1981). Qualitative histochemical tests have also shown DHP to be more concentrated in the outer portion of the root. Porter et al. (1976) measured phenolics with a nonspecific spectrophotometric method and found greater amounts associated with the periderm than with the central parenchyma of sweet potato roots. However, the tissue that was sampled was not clearly defined anatomically, and the phenolics were not separated into individual components.

The purpose of this study was (1) to investigate the cross-sectional and proximal (stem) end to distal (root) end distribution of phenolics in sweet potato roots (cv. Jewel) and (2) to identify and quantitate the individual phenolics with HPLC procedures using an inexpensive, two-chambered gradient device.

MATERIALS AND METHODS

Sweet potato roots (cv. "Jewel") were harvested, cured, and stored by using recommended procedures (Covington et al., 1976). Relatively blemish free roots of approximately the same shape and weight were removed from storage after 4 months, washed, air-dried at room temperature, and weighed prior to sectioning. Distribution studies were replicated 9 times with one root per replicate.

Cross-Sectional Distribution. Each replicate was halved midway between the proximal and distal ends of the root. Cross sections, 0.2 cm in thickness, were cut with a hand slicer until 50 g of tissue was obtained. Each cross-sectional slice was dissected with a razor blade into three groups of tissue: (1) periderm (skin); (2) secondary root tissue beneath the periderm and external to the cambium; (3) secondary root tissue internal to the cambium. These three groups of tissue were respectively designated as (1) skin, (2) outer tissue, and (3) inner tissue. Each of the three groups of tissue was collected, weighed, and blended with at least 4× its weight of boiling 95% ethanol. The ethanol extracts were held in the dark at 5 °C for 2 weeks before analysis.

End-to-End Distribution. Each weighed replicate was oriented with regard to proximal (stem) end and distal (root) end and then 1 cm of tissue was removed from each end and discarded. These discarded ends may have had abnormally high phenol content due to wounds inflicted during harvest. Ten-gram cross sections were then obtained from the stem end, root end, and middle portion by using a hand slicer. The skin was removed from the sections and discarded. Each section was then separated into outer and inner tissue and homogenized in ethanol, as described in the preceding paragraph.

Phenol Analysis. Each ethanol extract was suction-filtered through medium-fast filter paper. The mat was removed and mixed with 30–50 mL of 95% ethanol and refiltered. The filtrates were collected and the volume was reduced on a vacuum rotary evaporator (30 °C). The residue was removed from the flask with several small portions of water. The aqueous material was combined and diluted in a volumetric flask to the desired volume depending upon the sample weight. The aqueous samples were transferred to bottles and held at −10 °C until analyzed.

For measurement of the phenolic content, the samples were thawed and diluted in volumetric flasks with 0.1 M phosphate buffer (pH 6.25), and the absorbance at 323 nm (A_{323}) was measured. Ten milliliters of the diluted sample was shaken on a rotary shaker for 30 min at 50 rpm with

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

0021-8561/81/1429-0904$01.25/0

Table I. Phenols[a] Present in a Cross Section[b] of "Jewel" Sweet Potato Tissue

	phenols[c,d]							
tissue location	CAA	NEO	ISOA	ISOB	ISOC	UNK	total	%
skin	1.99^{A}	0.07^{C}	0.18^{A}	0.74^{A}	1.68^{B}	0.24^{A}	4.81^{B}	34.71^{B}
outer	2.04^{A}	0.68^{A}	0.23^{A}	0.87^{A}	2.41^{A}	0.06^{B}	6.29^{A}	43.47^{A}
inner	1.08^{B}	0.29^{B}	0.16^{A}	0.40^{B}	1.10^{C}	0.01^{B}	3.05^{C}	21.44^{C}
$LSD_{0.05}$	0.43	0.19	N.S.	0.24	0.56	0.05	1.37	8.63

[a] Milligrams as chlorogenic acid in the sections obtained from a 50-g slice of sweet potato. Mean of nine replicates with one root in each replicate. [b] Slices taken from midway between the proximal and distal ends of the roots. [c] CAA = chlorogenic acid; NEO = neochlorogenic acid; ISOA, -B, and -C = isomers of isochlorogenic acid; UNK = unknown peak. [d] In each column, values with the same letter are not significantly different at the $P < 0.05$ level.

0.2 g of purified, strong anion-exchange resin (Dowex 1-X8 or Rexyn-201). The A_{323} was again measured, and the phenol content (as chlorogenic acid) calculated from the change in absorbance. A series of chlorogenic acid solutions handled in the same way as the samples served as the standard (Walter and Purcell, 1979).

Those samples for which the distribution of isomers was needed were analyzed by HPLC. A 1- or 2-mL aliquot was mixed with 1 mL of coumarin internal standard (40 μg/mL). The mixture was passed through a C-18 cleanup column, and the phenols were eluted with a mixture of acetonitrile, methanol, and water (4:4:2 v/v/v) (Walter et al., 1979). The eluate was then injected onto the HPLC.

The phenolic identity was assigned from previous work (Walter et al., 1979) on the basis of commercially available standards and chemical analyses. The relative retention time (R_{RT}) for each phenolic was obtained by dividing the compound's retention time by the retention time of the internal standard. Quantification of the phenolics was accomplished by electronic integration of the peak areas, followed by application of the internal standard method to the areas (Walter et al., 1979). The concentration of each phenol was calculated as chlorogenic acid.

HPLC. The liquid chromatograph was a Waters ALC/GPC-204 unit equipped with a U6K injector, a μBondapak C-18 column (3.9 × 300 mm), and a Model 440 absorbance detector operating at 313 nm. This was a single pump unit which was adapted to generate a solvent gradient by connecting a two-chambered gradient elution device (GED) to the solvent intake line (Glenco Scientific, Houston, TX). The configuration of the GED was 2–50-mL chambers (2.4 × 11 cm) connected in sequence by a valve. The chamber connected directly to the pump (A) was equipped with a stirring bar and situated on a magnetic stirrer. Chamber A was filled with solvent and the system equilibrated to operate the gradient. During equilibration, 32 mL of solvent was placed in chamber B. The solvent in chamber A was adjusted to a volume of 30 mL, the sample was then injected, and the valve between the chambers opened. Chamber A was stirred vigorously during the entire analysis. For sweet potato phenolics, the best choice of starting solvent (chamber A) was 10% methanol–90% 0.033 M phosphate (pH 3.1; v/v) and final solvent (chamber B) was 60% methanol–40% 0.033 M phosphate (pH 3.1; v/v). The optimum flow rate was 1.5 mL/min and the analysis took ~33 min.

Statistical Procedures. The effect of section upon phenolic level was calculated by analysis of variance (ANOVA) and the Waller-Duncan K-ratio T test (SAS, 1979). Data treatment by these procedures provided least-significant differences (LSD) by which section means were compared.

RESULTS AND DISCUSSION

Phenolic Separation. The gradient HPLC method developed during the course of this research gave separation superior to the isocratic procedure previously used (Walter et al., 1979). The gradient partially separated chlorogenic (R_{RT} 0.63) from neochlorogenic acid (NEO; R_{RT} 0.67) and resolved isochlorogenic acid into ISOA, ISOB, and ISOC with R_{RT} values of 1.13, 1.18, and 1.22, respectively (Figure 1). In addition, an unidentified peak (UNK; R_{RT} 1.32) was observed in some of the samples.

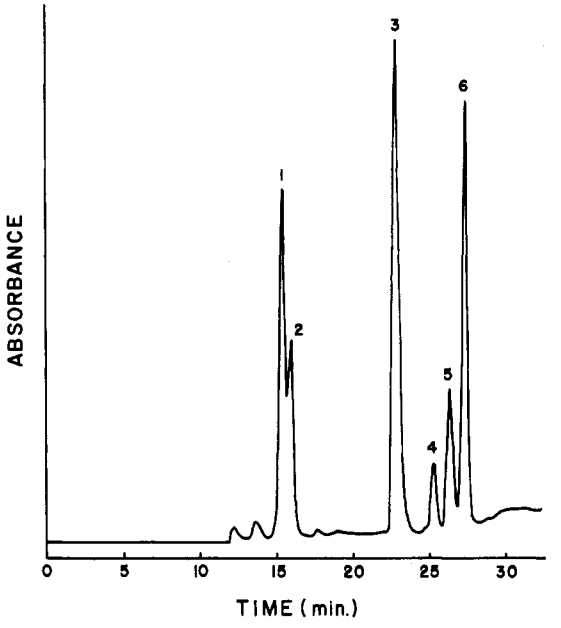

Figure 1. LC of phenolics from "Jewel" sweet potato (inner tissue). (1) Chlorogenic acid; (2) neochlorogenic acid; (3) coumarin (internal standard); (4) isochlorogenic acid A; (5) isochlorogenic acid B; (6) isochlorogenic acid C.

Cross-Sectional Distribution. Analysis of the relationship between phenolic content and tissue location revealed that phenolic content decreased in the order outer > skin > inner (Table I). In fact, the outer 5–6 mm of tissue (including the skin) contained 78.2% of the phenolics present. The only differences in this order are with NEO, which is significantly less abundant in the skin than in the other sections, and with the UNK, which is much more abundant in the skin than in the other sections. It is not known if these differences are due to variation in the skin itself or in the small amount of outer tissue adhering to the skin.

If the data are calculated on the basis of moles of quinic acid per mole of caffeic acid, it is seen (Table II) that the percent distribution of phenolics within each section is not significantly different with the exception of UNK. The monoisomers (1 mol of caffeic acid + 1 mol of quinic acid), CA and NEO (Sondheimer et al., 1961), compose ~43% of the total in each section, while the diisomers (2 mol of caffeic acid + 1 mol of quinic acid), ISOA, -B, and -C (Corse et al., 1965), make up ~55% of each section.

The preceding data were calculated on the basis of total phenolics within each section. It should be remembered that the skin comprised ~4% of the sample weight, the

Table II. Percent Distribution of Phenolics within Cross Sections

tissue location	mono-isomers[a,b]	diisomers[a,b]	UNK[b]
skin	42.83[A]	54.05[A]	4.99[A]
outer	43.24[A]	55.80[A]	0.95[B]
inner	44.92[A]	54.43[A]	0.33[B]
$LSD_{0.05}$	5.20	5.30	0.93

[a] Monoisomers are calculated from the sum of NEO and CAA; Diisomers are calculated from the sum of ISOA, ISOB, and ISOC. [b] In each column, values with the same letter are not significantly different at the $P < 0.05$ level.

Table III. Distribution of Phenolic Compounds in Sweet Potato Tissue Cross Sections

	phenols[a,b]			
tissue location	mono-isomer[c]	diisomer[c]	UNK[c]	total[c]
skin	100.53[A]	125.59[A]	10.58[A]	237.63[A]
outer	16.53[B]	21.37[B]	0.35[B]	38.24[B]
inner	3.95[B]	4.80[C]	0.04[C]	8.79[B]
$LSD_{0.05}$	17.38	16.37	2.21	33.00

[a] Milligrams per 100 g of tissue (as chlorogenic acid). [b] Monoisomers are calculated from the sum of NEO and CAA; diisomers are calculated from the sum of ISOA, ISOB, and ISOC. [c] In each column, values with the same letter are not significantly different at the $P < 0.05$ level.

outer portion ~35%, and the inner portion the remainder. When the data are expressed as milligrams of phenol per 100 g of tissue, the phenolic content decreased in the following order with respect to tissue location: skin ≫ outer ≥ inner (Table III). The skin contains 0.24% phenolics on a fresh weight basis, and the diisomers comprise 52.6% of this total. The inner tissue appears to contain less phenolics than does the outer tissue, but sample-to-sample variation was too large to detect any statistical differences (0.05 level).

End-to-End Distribution. The root and stem ends of the sweet potato are generally of significantly smaller diameter than the middle portion. Consequently, the amount of phenol-rich outer tissue makes up a higher part of the total weight at the two extremities. If phenolics were measured on an entire slice taken from either extreme, it would most likely reflect higher phenol content than the middle portion. Thus, we separated the tissue from stem end, root end, and middle portion into inner and outer tissue and measured the phenolic levels present in each.

Constituent analysis revealed that the end-to-end distribution of phenolic compounds was not significantly different (0.05 level) with respect to the portion of the root or tissue location within each portion. Thus, we have summarized the data and reported it as total phenol content (Table IV). Differences in the inner tissue phenol levels are not statistically significant (0.05 level). For the outer tissue, root end phenol levels are greater (0.05 level) than those of the midportion phenolics. If the data are recalculated on the basis of percent of total phenols in each section, then stem end and root end phenol levels are statistically higher (0.05 level) than are levels in middle portions. Similar results concerning phenolic distribution have been reported for white potato tissue (Reeve et al., 1969).

Table IV. End-to-End Distribution of Phenols[a] in Sweet Potato Tissue

	mg of CA/g of tissue[b]		% total phenolics[c]	
	inner	outer	inner	outer
root end	0.153[A]	0.418[A]	36.23[A]	39.49[A]
stem end	0.122[A]	0.390[A,B]	33.63[A]	36.67[A]
midportion	0.188[A]	0.239[B]	30.13[A]	23.83[B]
$LSD_{0.05}$	NS[d]	0.157	NS	11.75

[a] Results calculated from nine replicates with one root per replicate. [b] Phenols calculated as chlorogenic acid (CA). [c] In each column, values with the same letter are not significantly different at the $P < 0.05$ level. [d] NS, not significant.

Darkening of processed sweet potatoes is postulated to be the result of the interaction between polyphenol oxidase (PPO) and phenolic substrates. Work in this laboratory (Walter and Schadel, 1981) has shown that during normal lye peeling, heat penetration from the bath into the cambial area is sufficient to disrupt cellular organization but not great enough to inactivate PPO, thus causing the PPO–phenolic interaction. Data from the present study suggest that a more severe lye-peeling treatment which removed the outer 5 mm of tissue would also eliminate 78% of the substrate for darkening and thereby result in a product less prone to darkening.

LITERATURE CITED

Corse, J. W.; Lunden, R. E.; Waiss, A. C. *Phytochemistry* **1965**, *4*, 527.
Covington, H. M.; Wilson, L. G.; Averre, C. W.; Baird, J. V.; Sorensen, K. A.; Proctor, E. A. *N.C., Agric. Ext. Publ.* **1976**, *AG-09*.
Hoover, M. W. *Food Technol. (Chicago)* **1963**, *17*, 128.
Hughes, J. C.; Swain, T. *J. Sci. Food Agric.* **1962**, *13*, 358.
Porter, W. C.; Pharr, D. M.; Kushman, L. J.; Pope, D. T. *J. Food. Sci.* **1976**, *41*, 938.
Reeve, R. M.; Hautala, E.; Weaver, M. L. *Am. Potato J.* **1969**, *46*, 374.
SAS "SAS User's Guide"; SAS Institute: Raleigh, NC, 1979; pp 191–194.
Schadel, W. E.; Walter, W. M., Jr. *Can. J. Bot.* **1981**, in press.
Scott, L. E.; Appleman, C. O.; Wilson, M. *Md., Agric. Exp. Stn., Bull.* **1944**, *No. A33*.
Scott, L. E.; Kattan, A. A. *J. Am. Soc. Hortic. Sci.* **1957**, *69*, 436.
Sondheimer, E.; Szymanski, C. D.; Corse, J. W. *J. Agric. Food Chem.* **1961**, *9*, 146.
Walter, W. M., Jr.; Purcell, A. E. *J. Agric. Food Chem.* **1979**, *27*, 942.
Walter, W. M., Jr.; Purcell, A. E. *J. Agric. Food Chem.* **1980**, *28*, 941.
Walter, W. M., Jr.; Purcell, A. E.; McCollum, G. K. *J. Agric. Food Chem.* **1979**, *27*, 938.
Walter, W. M., Jr.; Schadel, W. E., submitted for publication in *J. Food Sci.*, 1981.

Received for review January 19, 1981. Revised manuscript received May 12, 1981. Accepted June 2, 1981. Paper No. 6755 of the journal series of the North Carolina Agricultural Research Service, Raleigh, NC. 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 the exclusion of other products that may be suitable.