Organic Acid Determination in Sweet Potatoes by HPLC

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An HPLC technique was developed for the analysis of individual organic acids in raw and baked sweet potato roots. Organic acids can be quantitated by isocratic elution with 0.0008 N H_2SO_4 as the mobile phase with a cation exchange resin column (Aminex HPX-87H) heated to 75 °C and ultraviolet detection at 214 nm. The major organic acids in both raw and baked roots were malic, citric, and succinic. Trace amounts of oxalic, oxaloacetic, and an unknown compound were also detected. Over 96% recoveries of all organic acids added to sweet potato roots were obtained.

Organic acids are important constituents of plant foods influencing flavor, stability, and keeping quality. They have been proposed as an index of maturity, ripeness, or spoilage in certain commodities. No information exists on individual organic acids in sweet potatoes and their role in the flavor of the processed product or physiology and keeping quality of the raw root. Organic acids are generated in the Krebs cycle during aerobic oxidation of carbohydrates, fats, and proteins in most biological systems.

Previous methods used for organic acid analysis include paper and thin-layer chromatography (TLC), ion exchange column chromatography, enzymology, and gas-liquid chromatography. Advances in high performance liquid chromatography (HPLC) systems including improved ion exchange resin columns have made this technique the preferred choice for many investigators requiring quantitative individual organic acid information. Organic acids in fruit juices and beverages (Palmer and List, 1973), food products (Turkelson and Richards, 1978), and dairy products (Marsili et. al., 1981) have been quantitated by HPLC. Schwarzenbach (1982) reviewed the separation systems applicable in HPLC for carboxylic acid analysis.

The objective of this study was to develop an HPLC procedure for the quantitation of individual organic acids in sweet potatoes.

MATERIALS AND METHODS

Samples. Six different sweet potato cultivars, Whitestar, Rojo Blanco, Centennial, Jewel, Jasper, and Travis, ranging from high to low dry matter, respectively, were evaluated for individual organic acid content in the raw and baked conditions. Baked roots were prepared by heating in a Magic Chef convection oven (American Stove Co., St. Louis, MO) for 75 min at 170 °C or in a microwave oven (Radarange, Amana Inc., Amana, IA) at 1500 W with a frequency of 2450 MHz for 15 min.

Preparation of Sweet Potatoes. Raw or baked unpeeled roots were longitudinally cut in half and uniformly grated over the entire surface to a depth of about 3 mm to obtain tissue representative of the whole root. Grated tissue from 6 different U.S. No. 1 grade roots was combined together to minimize root to root variability for each determination. A total of 8 determinations per cultivar were made. Exactly 10.00 g (sufficient for adequate resolution of organic acids with the HPLC system described below) of randomly selected tissue was homogenized in 80% ethanol to ensure rapid enzyme denaturation for 1 min at high speed with a Virtis 45 homogenizer. The resulting slurry was immediately boiled for 15 min, cooled, and filtered through Whatman No. 4 paper. The residue and original container were washed with additional 80% ethanol and made to a final volume of 100 mL. An aliquot of about 5 mL was filtered through a 0.45 μm Versapor membrane (Gelman Filtration Products, Ann Arbor, MI) to remove particulate impurities before injecting into the HPLC.

Preparation of Organic Acids Standards. Organic acid standard preparation followed the same procedure as sample preparation. Organic acid standards were prepared in the concentration ranges found in sweet potatoes. For a 0.5% standard of any organic acid, 0.500 g of analytical grade organic acid (Sigma Chemical Co., St. Louis, MO) was homogenized in 80% ethanol, boiled, filtered, made up to 100 mL with 80% ethanol, and ultrafiltered before injecting into the HPLC. Organic acids were not affected by heating, as checked against unboiled standards dissolved in 80% ethanol.

HPLC Analyses. A Beckman series 340 liquid chromatograph (Beckman Instruments, Inc., Berkely, CA) was used equipped with a Model 112 pump, Model 210 injector fitted with a 20-μL sample loop, and a Model 160 ultraviolet detector at a fixed wavelength of 214 nm. The detector was set at 0.100 AU and the peak area of the signal was electronically integrated by a Vista 401 integrator (Varian Assoc., Sunnyvale, CA) in the external standard mode by using an attenuation of 4 and a chart speed of 0.5 cm/min. Organic acids were separated with a 300 mm × 7.8 mm i.d. Aminex HPX-87H column (Bio-Rad Laboratories, Richmond, CA). Column temperature was maintained at 75 °C with an SSI column heater (Scientific Systems, State College, PA). Plumbed between the injector and the analytical column were respectively a 2-μL Rheodyne Model 7302 column inlet filter (Rheodyne, Cotati, CA) and a 40 × 4.6 mm ion exclusion guard cartridge packed with Aminex HPX-85H resin (Bio-Rad Laboratories). The mobile phase was degassed 0.0008 N H_2SO_4 made by diluting reagent grade concentrated sulfuric acid in HPLC grade water. Flow rate was 0.8 mL/min.

Recovery Studies. The percent recovery of each organic acid from both raw and baked roots was determined by weighing out two equal 10.00-g portions from the same root tissue; to one portion was added a known amount of organic acid (0.500 g of citric, 0.500 g of malic, 0.300 g of succinic, or exactly one-tenth this amount) and to the other portion nothing was added. The samples were prepared as described above. Percent recoveries were based on the difference between the total amount in the spiked vs. unspiked samples.

RESULTS AND DISCUSSION

Identification of each organic acid was based on HPLC retention times and confirmed by two-dimensional cellu-

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Table I. Recovery Study of Organic Acids Added to Sweet Potato Tissue

organic acid	amount in sweet potato, %	amount added, %	theoretical recovery, %	amount found, %	recovery, %
citric	0.281	0.050	0.331	0.324	98
	0.281	0.500	0.781	0.759	97
malic	0.222	0.050	0.272	0.262	96
	0.222	0.500	0.722	0.718	99
succinic	0.070	0.030	0.100	0.101	101
	0.070	0.300	0.370	0.359	97

lose TLC (Myers and Huang, 1969). Detector response to all organic acids was linear over the concentration range found within sweet potatoes. Less than 5% variability in individual organic acid concentration was found between triplicate injections of the same sample. Recoveries of all organic acids added to sweet potato tissue were over 96%. Recovery data for each organic acid of two different concentrations are presented in Table I.

Figure 1 illustrates a typical chromatogram of organic acid separation in a raw or baked sweet potato root using an Aminex HPX-87H column. The first peak is a mixture of water from the sample extract, phosphate, and other unretained compounds. This is closely followed by oxalic acid at 4.7 min. Oxalic acid strongly absorbs at 214 nm and even though the peak on the chromatogram is large the actual amount is very small. In the raw roots of all cultivars oxalic acid never exceeded 0.002%. The first major organic acid to elute is citric at 5.6 min. An oxaloacetic acid peak at 5.4 min immediately precedes citric acid only in baked roots. No oxaloacetic acid was found in raw roots and the concentration in baked roots never exceeded 0.02%. In some cultivars there is a small isocitric acid tail immediately after citric acid. The next major organic acid found in sweet potatoes is malic acid which elutes at 6.7 min. The sugars sucrose and fructose elute at 7.2 and 7.8 min, respectively. Maintaining a 0.0008 N H_2SO_4 mobile phase at a flow rate of 0.8 mL/min provides for adequate separation of malic acid and sucrose. Slightly increasing the normality to 0.001 N H_2SO_4 results in coelution of malic acid and sucrose and hence the impossibility of quantifying malic acid. Slightly decreasing the normality to 0.0005 N H_2SO_4 results in asymmetry and splitting of the citric acid peak. Even lower normalities split the malic acid peak. Succinic acid elutes at 8.8 min followed by an unknown peak at 10.8 min. An additional unknown peak at 12.6 min was detected only in Rojo Blanco roots. Neither of the unknown peaks were C_1–C_4 monocarboxylic acids or ascorbic acid. An asymmetric ethanol peak elutes at 17.0 min followed by very broad chlorogenic and caffeic acid peaks at 32 min and 52 min, respectively. Neither of these phenolic acid peaks are quantifiable, but due to their presence one must wait to reinject another sample until chlorogenic acid has eluted. Reinjections can be made after 32 min without overlap of caffeic acid from the previous sample with the organic acids of the next sample. A very flat base line exists between the two phenolic peaks.

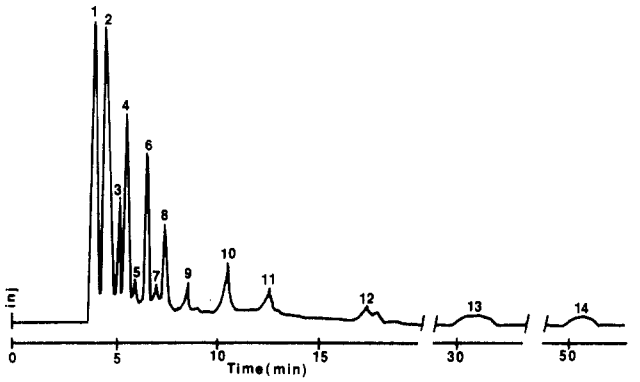

Figure 1. Typical chromatogram of organic acid separation in sweet potatoes on an Aminex HPX-87H column at 75 °C with 0.0008 N H_2SO_4 mobile phase at 0.8 mL/min, UV detection, and 20 μL injected. Peaks: (1) water; (2) oxalic; (3) oxaloacetic (only in baked roots); (4) citric; (5) isocitric; (6) malic; (7) sucrose; (8) fructose; (9) succinic; (10) unknown; (11) unknown (only in Rojo Blanco roots); (12) ethanol; (13) chlorogenic; (14) caffeic.

Concentrations of the three most abundant organic acids found in sweet potatoes are listed in Table II. Individual organic acid concentrations differed between cultivars. Citric and malic acids were the two most abundant organic acids found in raw and microwaved roots of all cultivars and generally the most abundant in convection oven baked roots. There was an inverse correlation between citric and malic acids among the cultivars tested. Those cultivars having the most citric acid usually ranked the lowest in malic acid concentration and vice versa. Citric acid increased in all cultivars during convection oven baking but not in microwaving. Malic and succinic acid increased in all cultivars during both convection oven and microwave baking, with the largest increases noted in the convection oven baking. The increased organic acid concentrations in oven baked or microwaved roots could partially be attributed to water loss during baking and microwaving. Approximately 18% weight loss occurred during baking and 28% during microwaving in each cultivar. The increases in each organic acid were generally greater than 18% during baking, indicating a net synthesis occurred. The changes in organic acids during the more dehydrating microwaving treatment were generally less than 28%, indicating there was no change or a net loss, as in the case of citric acid.

The Aminex HPX-87H column contains a 9 μm 8% cross-linked sulfonated polystyrene–divinylbenzene co-

Table II. Organic Acid Concentration in Raw and Baked Roots of Six Different Sweet Potato Cultivars Held at 15.6 °C for 3 Months[a]

	% organic acid (fresh weight basis)								
	citric			malic			succinic		
cultivar	raw	oven[b]	MW[c]	raw	oven	MW	raw	oven	MW
Whitestar	0.35[d]	0.45	0.38	0.10	0.14	0.11	0.06	0.15	0.08
Jewel	0.28	0.38	0.30	0.22	0.30	0.28	0.07	0.19	0.09
Travis	0.19	0.22	0.18	0.24	0.34	0.33	0.08	0.21	0.08
Rojo Blanco	0.16	0.19	0.14	0.23	0.33	0.28	0.07	0.19	0.09
Centennial	0.15	0.20	0.15	0.33	0.41	0.42	0.08	0.29	0.11
Jasper	0.14	0.19	0.15	0.37	0.48	0.45	0.08	0.21	0.09

[a] Less than 0.002% oxalic acid was found in all roots. Oxaloacetic acid was detected only in processed roots. Two unknown peaks, 10.8 min in all roots and 12.6 min in Rojo Blanco roots, were also detected. [b] Baked in convection oven at 170 °C for 75 min. [c] Baked in microwave oven at 1500 W, 2450 MHz for 15 min. [d] Each value represents a total of 48 roots (8 different determinations × 6 roots per determination).

polymer resin in the H^+ form. The 0.0008 N H_2SO_4 mobile phase continuously regenerates the resin H^+ form during operation. Separation of organic acids occurs by ion exclusion and partitioning and organic acids elute in order of increasing pK_a (Jupille et. al., 1981). Heating the column to 75 °C speeds up analysis time and increases peak resolution. The function of the small ion exclusion guard cartridge is to bind irrevesibly the positively charged inorganic ions which would otherwise bind to the analytical column resin decreasing its separation capability of organic acids. Highly charged negative ions are excluded from the analytical column resin and elute in the void volume (Bio-Rad, 1981). The Aminex HPX-87H column has a long useable lifespan. More than 800 injections were made over a period of one year without any noticeable deterioration in separation capabilities.

Ultraviolet detection at 214 nm was able to detect organic acid concentrations less than 0.002%. An alternative method of detection, refractive index, was tried but resulted in several times less organic acid sensitivity.

Registry No. Oxaloacetic acid, 328-42-7; isocitric acid, 320-77-4; sucrose, 57-50-1; fructose, 57-48-7; ethanol, 64-17-5; chlorogenic acid, 327-97-9; caffeic acid, 331-39-5; maleic acid, 110-16-7; citric acid, 77-92-9; succinic acid, 110-15-6; oxalic acid, 144-62-7.

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Purification of Deoxynivalenol (Vomitoxin) by Water-Saturated Silica Gel Chromatography

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A simple procedure was developed for the laboratory production and purification of gram quantities of crystalline deoxynivalenol (DON). When *Fusarium graminearum* R6576 was grown on rice, concentrations of 600–700 ppm DON were obtained after 13–18 days of incubation. A DON derivative, 15-acetyl-DON (15-ADON), was also found at concentrations of 100–300 ppm after 7–10 days. Crude culture extracts were purified by low pressure liquid chromatography on a column of water-saturated silica gel which selectively extracted DON when methylene chloride was used as the mobile phase. After elution of DON with water and subsequent reextraction with ethyl acetate, DON could be readily crystallized. Purity of crystallized DON was verified by thin layer and high performance liquid chromatography.

Deoxynivalenol (3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one, DON), also known as vomitoxin, is a trichothecene mycotoxin produced by *Fusarium graminearum*, which has been associated with vomiting and feed refusal in swine (Vesonder et al., 1976; Forsyth et al., 1977). Recent examination of *Fusarium* infected grains, particularly in Canada and the Midwest, has confirmed the natural occurrence of DON (Scott et al., 1981; Eppley et al., 1984; Trenholm et al., 1983). Assessment of the hazards associated with exposure to DON has been hampered by a lack of the gram quantities of pure DON required for toxicological studies.

An efficient method for DON production requires a convenient, concentrated source of DON. Whereas concentrations found in naturally contaminated grains are not high enough for use of this material as a source, inoculation of growing corn with DON-producing *Fusarium* strains has provided a concentrated source of DON (Miller et al., 1983; Scott et al., 1984). DON production in lab culture provides a more convenient source of crude DON and both solid and liquid substrates have been investigated (Vesonder et al., 1982; Greenhalgh et al., 1983; Greenhalgh et al., 1984). Although several multistep schemes for purifying crude DON extracts involving solvent–solvent partitioning, column chromatography, preparative thin layer chromatography (TLC), and high pressure liquid chromatography (HPLC) have been reported (Pathre and Mirocha, 1978; Bennett et al., 1981; Ehrlich and Lillehoj, 1984; Scott et al., 1984), these have the disadvantage of requiring numerous, time consuming chromatography steps.

The results reported here describe an efficient method for producing and purifying gram quantities of crystalline DON on a laboratory scale. DON was produced at very high levels by *Fusarium graminearum* R6576 on rice and was purified in a single step by low pressure liquid chromatography using a water-saturated silica gel column that is selective for DON.

MATERIALS AND METHODS

Inoculum Preparation. Potato dextrose agar plates were inoculated from stock soil cultures of *Fusarium graminearum* R6576 (*Gibberella zeae* U5373), a Michigan wheat isolate previously designated as W-8 (Hart et al., 1982), and incubated at 25 °C for 7 days in a 12-h light/dark cycle. Agar plugs (4 mm) removed from the growing edge of colonies were added to 500-mL Erlenmeyer flasks (3–4 plugs per flask) containing (carboxymethyl)-cellulose (CMC) medium (90 mL) (Cappellini and Peterson, 1965). These flasks were agitated on a rotary shaker (250 rpm) at 25 °C for 3–5 days. The suspension was

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