Application of Anion-Exchange High-Performance Liquid Chromatography in Determining Oxalates in Taro (*Colocasia esculenta*) Corms

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A recently introduced strong anion-exchange column (Alltech Universal Anion) was tested for measuring oxalate contents in taro (*Colocasia esculenta*) corms. The column was developed with a mobile phase of 4 mM phthalic acid with its pH adjusted to 4.5 using lithium hydroxide. The flow rate was 1.0 mL/min. The system is compatible with a conductivity detector. Compared with two previously published HPLC methods, this method is preferable in terms of simplicity and accuracy. Total oxalates and soluble oxalates were measured in 1 N HCl and water extracts, respectively. Insoluble oxalate contents were the differences between them by calculation. In nine taro cultivars, total oxalate contents ranged from 43 to 156 mg/100 g of fresh weight and soluble oxalate contents ranged from 19 to 87 mg/100 g of fresh weight. Insoluble oxalate contents were calculated to be 29.35–73.97% of the total oxalate contents in tested taro corms.

INTRODUCTION

Oxalic acid is a strong chelating agent widely distributed in crop plants. This dicarboxylic organic acid forms water-soluble salts with sodium, potassium, or ammonium. It may also exist as insoluble crystals of calcium oxalate (Libert and Franceschi, 1987). The deposition of calcium oxalate crystals in kidney may result in renal damage (Holloway et al., 1989). The presence of oxalate in foods reduces the bioavailability of calcium (Weaver et al., 1987). In tropical root crops, particularly taro and elephant foot yam, calcium oxalate is present as fine needlelike raphides. The occurrence of these crystals has been considered as one of the causes of acridity in these crops (Tang and Sakai, 1983). Consequently, it is important to develop a reliable yet rapid method of testing soluble and insoluble oxalates in crop plants.

High-performance liquid chromatography (HPLC) has become the method of choice in measuring nonvolatile compounds like organic acids. There were mainly two HPLC column systems proposed and tested in the past for the direct measurement of oxalic acid. One included an ion-exclusion column and a mobile phase of dilute sulfuric acid (Picha, 1985; Holloway et al., 1989). The other one applied the ion-coupling mechanism on a reversed-phase partition column (Libert, 1981; Howe et al., 1990). These HPLC procedures are favored for their speed and simplicity in sample preparation. They are also more precise than the classical precipitation method. Because of the high equivalent conductance of the eluents, however, both systems can only employ a low-wavelength UV detector instead of the more specific conductivity detector to monitor and quantify oxalic acid. Low detector selectivity makes these methods vulnerable to interferences from other natural components such as acids and aldehydes to an accurate quantification of oxalic acid (Schwarzenbach, 1982).

The purpose of this study was to develop a new direct HPLC method in quantifying oxalic acid, which is compatible with the more selective conductivity detector. This method was compared simultaneously with one direct HPLC method and one indirect HPLC method involving enzymatic treatment and derivatization of oxalic acid (Murray et al., 1982; Martz et al., 1990). Furthermore, the soluble and insoluble oxalate contents in nine cultivars of the Hawaiian-grown taro (*Colocasia esculenta*) were determined and compared.

MATERIALS AND METHODS

Sample Preparation. The taro crops were harvested in late August at the University of Hawaii experimental farm. A brief description of the nine cultivars is given in Table I. The corms were peeled and then sliced into 5 mm thick sections. The samples were weighed and then freeze-dried. A subsample was dried at 100 °C to constant weight to determine the percent moisture in corms. The freeze-dried sample was ground to a fine powder in an electric grinder.

Soluble and insoluble oxalates were extracted with distilled water and 1 N HCl, respectively (Libert and Franceschi, 1987). The extraction started with mixing 1 g (weighed to 0.1 mg) of the dried powder with 15 mL of the liquid in a 50-mL Erlenmeyer flask. The flask was then tightly capped and placed in a boiling water bath for 18 min. After cooling to room temperature and making up volume with distilled water in a 50-mL volumetric flask, the mixture was filtered through a Whatman No. 542 filter paper. Extraction with hot HCl gave total oxalates, which included water-soluble oxalates and calcium oxalates. The water extraction gave the water-soluble oxalates. At least five replications were performed for each cultivar sample. The filtrate was again filtered through a 0.45-μm membrane filter prior to HPLC analyses.

Direct Measurement with HPLC. The chromatography was performed with a SP8800 programmable isocratic solvent delivery unit (Spectra-Physics, San Jose, CA) equipped with a variable-wavelength UV detector (Spectra 100, Spectra-Physics) and a LDC conductomonitor (Milton Roy, Riviera Beach, FL). The sample was injected through a Rheodyne 7125 loop injector that was fitted with either a 20- or a 100-μL sample loop. Chromatograms were recorded and peak areas were analyzed using a digital integrator (Shimadzu CR601). There were two column systems used. The Aminex column system was first used by Picha (1985) and then modified by Holloway et al. (1989). The system included an ion-moderated partition column (Aminex HPX-87H, 300 × 7.8 mm, Bio-Rad Laboratories, Richmond, CA) and a mobile phase of 0.0125 M H_2SO_4. Triplicate 20-μL samples of each extract were injected. The flow rate was 0.5 mL/min, and the UV detector was set at 214 nm.

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Table I. Description of Nine Different Cultivars of Taro (*C. esculenta*)[a]

cultivar	height[b]	maturity, months	characteristics
Eleele Makoko	M	8–12	light purplish-black petioles and light lilac-purple corm flesh
Haokea	M	9–12	light self-green petioles and narrowly ovate leaf blades
Lauloa Palakea	T	8–12	petioles have conspicuous black edges
Lehua Keokeo	M	8–12	pale green petioles with broad purplish-black edges, known to be less acrid
Manini Opelu	M to T	9–12	profuse light and dark green striping of the petiole, with reddish tinge on upper third
Moi	M	9–12	light green petioles with pinkish base
Piko Eleele	M	12–15	dark purplish petioles
Piko Ulaula	M	15–18	lilac-purple corm flesh
Pololu	M	12–15	white petiole base and light reddish-brown petioles, diffused with yellowish green

[a] Summarized from Whitney et al. (1939). [b] M, medium; T, tall.

The Alltech column system included a strong anion-exchange column (Universal Anion, 150 × 4.6 mm, Alltech Associates Inc., Deerfield, IL) and a mobile phase of 4 mM phthalic acid (adjusted to pH 4.5 with lithium hydroxide). Acid extracts were treated with a cation-exchange cartridge in the Ag^+ form (IC-Ag Maxi-Clean cartridge, Alltech Associates) prior to HPLC analysis. The cartridges were preconditioned by rinsing the packing with 8 mL of distilled deionized water. Three milliliters of filtered acid extracts were then passed through the cartridge at 1 mL/min. The first 1 mL of sample exiting the cartridge was discarded, and the next 2 mL was collected for injecting onto the HPLC column. Triplicate 100-μL samples of each filtrate were injected. Conductivity of the eluates was monitored.

The quality of HPLC separation was evaluated by the capacity factor k' and the resolution factor R. The capacity factor of oxalic acid peak was calculated as

$$k' = (V_1 - V_0)/V_0 \quad (1)$$

where V_0 and V_1 were the void volume of the column and the retention volume of oxalic acid, respectively. The resolution factor of any two peaks was calculated as

$$R = 2(V_2 - V_1)/(W_1 + W_2) \quad (2)$$

where V_1 and V_2 were retention volumes of respective peaks and W_1 and W_2 were peak widths measured at base line.

Measurement of *o*-Phenylenediamine-Derivatized Oxalate. We closely followed the procedure reported by Martz et al. (1990) as the reference method to compare the other two HPLC methods used. The procedure included a derivatization step in which sample solutions were mixed with 0.1 M *o*-phenylenediamine to convert oxalate to 2,3-dihydroxyquinixaline (DHQ). In blanks, the oxalate was removed by oxalate decarboxylase (EC 4.1.1.2). The enzyme solution was prepared by diluting 1 mL of sodium acetate (pH 4) containing 4 EU of oxalate decarboxylase to 10 mL with distilled water. The incubation condition was 16 h at 37 °C. All solutions were then fractionated with disposable C-18 cartridges, followed by a HPLC analysis on a C-8 column (Maxsil 5μ, Phenomenex, Torrance, CA) with doubly distilled water as the eluent. The UV detector was set at 314 nm to monitor DHQ.

Data Analysis. Five replicate determinations of oxalic acid content were averaged to obtain a single datum point for each taro sample by a set extraction (water vs acid) and HPLC quantification procedure. The significance of differences in averaged oxalic acid contents in each of the nine taro cultivars as determined by three different HPLC methods was analyzed using Duncan's multiple range test in the STATPAK statistical software program (Northwest Analytical Inc., Portland, OR).

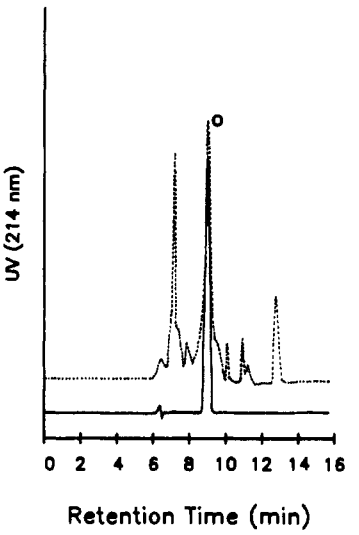

Figure 1. HPLC chromatograms of oxalic acid standard (solid line) and an acid extract of taro corms. Isocratic separation employed a mobile phase of 0.0125 M H_2SO_4 at a flow rate of 0.5 mL/min on an Aminex HPX-87H column (300 × 7.8 mm). o, oxalate.

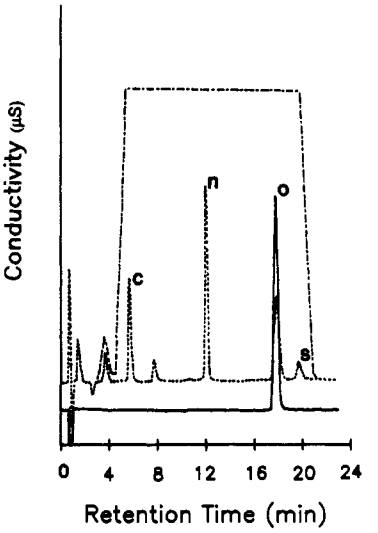

Figure 2. HPLC chromatograms of oxalic acid standard (solid line) and a HCl acid extract of taro corms before (broken line) and after (dashed line) a cation-exchange cartridge treatment. Chromatograms were developed by a mobile phase of 4 mM phthalic acid (adjusted to pH 4.5 by lithium oxide) at a flow rate of 1.0 mL/min on a Universal Anion column (150 × 4.6 mm). Conductivity was measured by a LDC conductomonitor in micro-Siemans (μS). c, chloride; n, nitrate, o, oxalate; s, sulfate.

RESULTS AND DISCUSSION

Direct HPLC Analyses. Both Aminex HPX-87H and Alltech Universal Anion columns can retain oxalic acid (Figures 1 and 2). The oxalic acid peak was identified by the co-injection of internal standards. Its identity was further confirmed by oxalate decarboxylase, which removed the standard peak. The capacity factor k' of oxalic acid was 0.4 on the Aminex column and 14.1 on the Alltech column. A k' of 0 means that the sample comes through the column unretained. Due to the small degree of retention on the Aminex column, there were other UV positive compounds in taro extracts which eluted simultaneously or very close to oxalic acid. Holloway et al. (1989) also noticed the interferences in some of the tropical crops

they analyzed and tried to avoid them by increasing the acid concentration of eluent. We found that some of the interferences in taro corms could indeed be eliminated by changing the temperature and concentration of the mobile phase, but there was still some interference with the separation of oxalic acid (Figure 1). We tried to use a conductivity detector to monitor the eluates but failed due to the strong conductivity of sulfuric acid in the eluent. The lack of selectivity of using UV detectors in monitoring organic acids has been previously discussed (Schwarzenbach, 1982).

This is the first time that the Alltech Universal Anion column has been reported in measuring oxalic acid. The stationary phase of the Alltech Universal Anion column includes a copolymer matrix of 2-hydroxyethyl methacrylate and ethylene dimethacrylate. It is extensively crosslinked to produce a matrix with a high chemical and physical stability (Vlacil and Vins, 1987). The excess hydroxyl groups on the matrix also make it more hydrophilic than the other poly(styrenedivinylbenzene)-based anion exchangers. Commonly used eluents that are compatible with a conductivity detector include *p*-hydroxybenzoic acid, phthalic acid, and gluconate/borate (Saari-Nordhaus et al., 1991). Each mobile phase seems to work equally well for the separation of inorganic acids like hydrochloric acid or nitric acid. For the separation of oxalic acid in this Alltech column, however, the mobile phase of phthalic acid at pH 4.5 was preferred for the clear resolution between oxalic acid and sulfuric acid (Figure 2). The divalent sulfate was the major interference to oxalic acid in the Alltech column, probably due to their similarities in hydrophilicity, molecular weight, and ionic charge. The resolution factor R between the acids increased from 0.8 in the *p*-hydroxybenzoic acid mobile phase (pH 7.9) to 2.4 in the phthalic acid mobile phase (pH 4.5). We speculated that at the lower pH the second carboxylic acid functional group in oxalic acid was not fully ionized ($pK_2 = 4.3$); therefore, the differences in anionic charge between oxalic acid and sulfuric acid increased and so did the resolution.

The k' value of oxalic acid in the Alltech column was greater than the generally considered optimal value of between 2 and 6. Nevertheless, the time required to complete an analysis was approximately the same for the Aminex column and the Alltech column. This was because the Alltech column functioned at a higher flow rate, while there were more compounds eluted after the oxalate in the Aminex column. Even with a much greater k' factor, the width of the oxalate peak in the Alltech column did not increase substantially. This was attributed to the high hydrophilicity of the column matrix (Vlacil and Vins, 1987). The average recovery of oxalic acid added to taro samples was 96%, with a coefficient of variability ranging from 2 to 4%.

Removal of Chloride Ions. When hydrochloric acid was used to extract water-insoluble calcium oxalate, the excessive amount of chloride ions was removed by cation-exchanger cartridge in the Ag^+ form before the extract was injected onto an Alltech Universal Anion column. The chloride ions were precipitated through formation of insoluble AgCl inside the cartridges. As shown in Figure 2, without the cartridge treatment, the chloride ions in HCl acid extracts overloaded the anion column and masked subsequently eluted compounds, including oxalic acid. With the cartridge treatment of the extracts, however, only a small amount of residual chloride ions was left which no longer interfered with the quantification of oxalic acid. The solubility of silver oxalate in water is approximately 20 times greater than the solubility of AgCl. The recovery rate of oxalic acid in 1 N HCl through the cartridge treatment was between 96 and 102% when the concentration of oxalate was less than 400 ppm. None of the taro extracts had total oxalate exceeding 300 ppm.

Table II. Total and Soluble Oxalate Contents (Milligrams/100 g of Fresh Weight) in Taro Corms of Nine Cultivars Determined by Three Methods

	HPLC methods[a]		
cultivar	Aminex column	Alltech column	derivatized oxalate
Eleele Makoko			
soluble	62 ± 4[b]	45 ± 3	51 ± 5
total	105 ± 8[b]	82 ± 6	77 ± 8
Haokea			
soluble	74 ± 5	65 ± 4	68 ± 7
total	122 ± 8[b]	92 ± 7	98 ± 8
Lauloa Palakea			
soluble	62 ± 4	53 ± 3	59 ± 5
total	86 ± 6	72 ± 4	78 ± 8
Lehua Keokeo			
soluble	67 ± 5	58 ± 6	54 ± 7
total	121 ± 8	108 ± 7	102 ± 8
Manini Opelu			
soluble	43 ± 4	38 ± 2	39 ± 4
total	122 ± 5[b]	88 ± 6	98 ± 8
Moi			
soluble	48 ± 5	37 ± 2	38 ± 4
total	211 ± 9[b]	98 ± 6	91 ± 8
Piko Eleele			
soluble	87 ± 6[b]	56 ± 4	48 ± 6
total	210 ± 20[b]	146 ± 7	150 ± 10
Piko Ulaula			
soluble	127 ± 8[b]	87 ± 6	90 ± 10
total	190 ± 10[b]	156 ± 8	150 ± 10
Pololu			
soluble	35 ± 3[b]	19 ± 2	15 ± 3
total	54 ± 4[b]	43 ± 2	41 ± 4

[a] Average of five measurements ± SD. [b] A result significantly different ($p < 0.05$) among the three methods.

Oxalates Measured by Different Methods. The total and soluble oxalates in taro corms of nine varieties were measured with the two direct HPLC methods and a third method that measured the derivatized oxalates (DHQ). The third method is more complicated and time-consuming but is considered to be less affected by interferences (Martz et al., 1990). Results of the measurements are listed in Table II. With a few exceptions, the Aminex column method yielded significantly higher ($p < 0.05$) oxalate contents than the other methods. The results from the Alltech column method generally agreed with those from the derivatized oxalate method. The high oxalate values yielded by the Aminex column method indicated the effects of interferences to oxalate separation and detection. As we discussed previously, when these interferences cannot be eliminated, overestimation of oxalates would occur. The interferences seemed to be less in measuring soluble oxalates, where five of nine cultivar samples had similar measurements by all three methods. In contrast, seven of nine cultivar samples had significantly higher total oxalate measurements by the Aminex column method. The largest deviation occurred in the Moi taro extract, where the Aminex column method yielded total oxalate contents more than twice the amounts determined by the other two methods. It is speculated that one or more acid-extractable and UV-detectable components in some taro cultivars are the main interferences to oxalate separation on the Aminex column. On the basis of this comparison, the direct HPLC method employing an Alltech Universal Anion column is a fast yet reliable way of analyzing total and soluble oxalates in taro corms.

Table III. Insoluble Oxalate Contents (Milligrams/100 g of Fresh Weight) in Taro Corms

cultivar	insoluble oxalate[a]	% insoluble oxalate of total oxalate
Eleele Makoko	37 ± 3^{b}	45.12
Haokea	27 ± 3^{a}	29.35
Lauloa Palakea	$29 \pm 2^{a,b}$	40.28
Lehua Keokeo	$50 \pm 4^{b,c}$	46.30
Manini Opelu	70 ± 3^{c}	56.82
Moi	61 ± 2^{c}	62.24
Piko Eleele	108 ± 4^{d}	73.97
Piko Ulaula	69 ± 4^{c}	44.23
Pololu	24 ± 1^{a}	55.81

[a] Mean ± standard error; common superscript letter within a column indicates nonsignificance ($p < 0.05$).

Insoluble Oxalate Content and Acridity. Tang and Sakai (1983) concluded that the acridity in taro can be related to two general sources: (1) physical localized raphide irritation and (2) widespread irritating chemical(s) associated with the raphides. On the basis of their conclusion, the calcium oxalate raphides are closely related to, if not the direct cause of, taro acridity. We calculated the water-insoluble calcium oxalate contents by subtracting water-soluble oxalate contents from respective total oxalate contents. Values determined by the Alltech column method were used in calculation. The standard errors of the insoluble oxalate contents were calculated as

$$s = \sigma(1/n_A + 1/n_B)^{0.5} \quad (3)$$

where σ was estimated by the pooled internal standard deviations of total and soluble oxalate contents and n_A and n_B are replications of each oxalate measurement (n_A and $n_B = 5$). Results of nine analyzed taro cultivars are given in Table III. Statistically significant differences in insoluble oxalate contents existed among tested taro cultivars. The oxalate contents ranged from an average of 24 mg/100 g in the Pololu cultivar to an average of 108 mg/100 g in the Piko Eleele cultivar, on a fresh weight basis. The percentage of insoluble calcium oxalate of the total oxalate also varied from less than 30% to over 70%. It is evident that the bioavailability of calcium in the insoluble calcium oxalate is greatly reduced (Weaver et al., 1987). For the same cultivar, the oxalate contents were quite consistent, as indicated by the standard error of the means. However, since those taro plants were grown and harvested at the same time at the experimental farm, we could not examine the impact of plant maturity and variation in agronomy practices on oxalate contents.

In cultivars known to be low in acridity, such as Haokea, Lauloa Palakea, and Pololu, our results of the insoluble oxalate contents were low as well. The Piko cultivars are known to be very acrid; their insoluble oxalates were determined to be high (Table III). Noted exceptions to this were the Haokea and Lehua Keokeo cultivars. The Haokea taro was known to be high in acridity, and the sample we tasted was very irritating, but the insoluble oxalate was low. To the contrary, the Lehua taro was tasted low in acridity, but the insoluble oxalate contents were high. Most of the knowledge on taro acridity was not well documented, and further studies on this subject are warranted. A reliable and rapid HPLC method, as the one developed here, would be useful in the immediate future studies.

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