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Organic Acids and Calcium Oxalate in Tropical Root Crops

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Oxalate, malate, citrate, and succinate contents of tropical root crops were determined by HPLC. Water extraction gave soluble oxalates, and extraction with acid gave total oxalates. The difference between them equaled the amount of calcium oxalate. Total calcium was determined by atomic absorption, and free calcium (calcium not present as calcium oxalate) was readily calculated. Taro (*Colocasia esculenta*) leaves, from edible and nonedible cultivars (because of their acrid nature), showed no differences in their amounts of total oxalate or of calcium oxalate. This showed that acridity of taro leaves was not due solely to calcium oxalate raphides present. Stems of giant swamp taro (*Cyrtosperma chamissonis*), elephant foot yam (*Amorphophallus campanulatus*), skin of giant taro (*Alocasia macrorrhiza*), and taro leaves contained about 400 mg/100 g fresh weight of calcium oxalate, about 10 times the amount present in sweet potato, cassava, taro *Colocasia* and *Xanthosoma*, and yam. The free calcium content was 0–20 mg/100 g fresh weight and would be adequate for all root crops, except taro *Xanthosoma*.

Oxalate is widely distributed in plants in a readily water-soluble form as potassium, sodium, and ammonium oxalate and as insoluble calcium oxalate (Fassett, 1973; Connor, 1977; Smith, 1982). Since calcium may also occur in plants other than as insoluble calcium oxalate crystals, the mole ratio of oxalate to calcium is found to vary from 7 to <1 (Fassett, 1973). In tropical root crops and particularly in the aroids, viz. taro *Colocasia*, taro *Xanthosoma*, giant taro, giant swamp taro, and elephant foot yam, calcium oxalate is present as fine needlelike crystals or raphides (Sakai and Hanson, 1974; Sunell and Healey, 1979; Nixon, 1987; Bradbury and Holloway, 1988). The occurrence of these crystals has been considered as either the cause or a contributing cause of the acridity of some species of taro, giant taro, and giant swamp taro (Sakai and Hanson, 1974; Tang and Sakai, 1983), which causes irritation to the skin and swelling of the mouth and throat.

The two main toxic effects of oxalate poisoning are (1) acute poisoning, resulting in hypocalcaemia after ingestion of high levels of soluble oxalates, and (2) (more commonly) chronic poisoning in which calcium oxalate crystals are deposited in the kidneys, resulting in renal damage (Connor, 1977). The presence of oxalate in foods has also been implicated in reducing the bioavailability of essential minerals such as calcium (Kelsay, 1985). We have developed a method for the determination of calcium oxalate as well as water-soluble oxalate and other organic acids. The methods were applied to the tropical root crops of the South Pacific.

MATERIALS AND METHODS

Root crops were harvested in Papua New Guinea (PNG), Solomon Islands, Fiji, Western Samoa, Kiribati, Tonga, and Ponape State of the Federated States of Micronesia and cleaned, and the fresh weight was recorded. They were sent by air freight to Canberra and were stored at 15 °C

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until processed, normally within 1 week of harvesting. The root was weighed and peeled, and for the smaller specimens the whole root was used. For larger samples, three cross-sectional slices each of about 100 g were taken from either end and the center of the root or stem and used as one sample. The sample was weighed and then dried at 40 °C until a constant dry weight was obtained. A subsample was taken and dried at 100 °C to constant weight to obtain the percent moisture in the root crop. The sample dried at 40 °C was ground to a fine powder in an electric grinder.

With 30-mL-capacity glass-stoppered test tubes, 1 g of the dried powder was added to either 25 mL of distilled water or to 25 mL of 0.25 M H_2SO_4, and 1 mL of the internal standard (10 g of glutaric acid in 100 mL of water) was added. The mixture was placed in a boiling water bath for 10 min, cooled, and made up to volume in a 100-mL standard flask. A small volume of solution was filtered through a No. 542 filter paper, and this filtered solution was then again filtered through a 0.45-μm Acrodisc before separation by HPLC, by a modified procedure of Picha (1985). A 300 mm × 7.8 mm ion-exclusion column (HPX-87H) was used with 0.0125 M H_2SO_4, at a flow rate of 0.5 mL/min and a UV detector operating at 214 nm. For calibration of the HPLC system, a standard mixture of sodium oxalate (0.02 g), malic acid (0.02 g), sodium citrate (0.02 g), succinic acid (0.02 g), and glutaric acid (0.08 g) in 100 mL of 0.0125 M H_2SO_4 was used. The suitability of the procedure was substantiated by obtaining a greater than 98% recovery of known quantities of added oxalic acid.

Extraction with boiling dilute H_2SO_4 gave total oxalates, which included water-soluble oxalates plus calcium oxalate. The water-soluble oxalates (potassium and sodium oxalates and oxalic acid) were determined after extraction with boiling water followed by cooling the solution. Wills et al. (1983) used cold water, and Picha (1985) utilized 80% ethanol for this purpose.

The calcium determination was made as follows. A weighed amount of root crop dried at 40 °C was ashed in a furnace at 500–550 °C for about 16 h. The ash was dissolved in 5 mL of 3 M HCl and the resultant mixture diluted to 25 mL with 5 mL of a lanthanum oxide solution (5000 ppm) and water. The concentration of calcium in solution was determined by atomic absorption spectroscopy using a Varian 1275 instrument calibrated against freshly prepared standard solutions of calcium.

The treatment of the data from the determinations of total oxalate (sulfuric acid extraction), water-soluble oxalate, and total calcium was as follows: total oxalate (g) = water-soluble oxalate (g) + oxalate (g) present as calcium oxalate (CaOx), where calcium oxalate (g) = (total oxalate − soluble oxalate) × 128/88. And, total calcium (g) = free calcium (g) + calcium (g) present in CaOx, where free calcium (g) = total calcium (g) − (calcium oxalate × 40/128). Free calcium was considered to be calcium not combined as insoluble calcium oxalate.

RESULTS AND DISCUSSION

The retention times given in Table I for a range of organic acids showed that it was possible to obtain a reasonable separation of oxalic, citric, malic, and succinic acids present in the root crops. The presence of each of these acids was checked by running the chromatogram from the root crop, which was repeated after spiking with an authentic sample of each of the organic acids in turn. The intensity of each of the HPLC peaks was increased in turn by the addition of authentic material. In some cases additional unidentified peaks occurred in the HPLC trace.

Table I. Retention Times (min) of Organic Acids on an Ion-Exclusion Column HPX-87H Using H_2SO_4 as Eluent[a]

organic acid	0.0125 M	0.004 M
acetic acid		18.1
aconitic acid		11.9
butyric acid		22.0
citric acid	10.5	9.5
formic acid		16.4
glutaric acid	19.4	18.3
glycolic acid		14.6
glyoxylic acid		11.0
malic acid	12.5	11.5
methylmaleic acid		12.5
oxalic acid	9.8	7.5
succinic acid	15.6	14.8
tartaric acid		10.2

[a] Initially 0.004 M H_2SO_4 was used but with some of the root crop samples an unidentified peak occurred before oxalate that overlapped with it. It was possible by use of 0.0125 M H_2SO_4 to delay the oxalate peak sufficiently to obtain a good separation.

Table II. Variation of Oxalate Content (Milligrams/100 g Fresh Weight) across the Corm for Giant Taro and Taro *Xanthosoma*

sampling posn across radial sect	giant taro[a]			taro *Xanthosoma*: total oxalate[b]	
	total oxalate	sol oxalate	calcium oxalate	cv. 1	cv. 2
skin	310	ND[c]	451		
1 cm below skin	135	10	182	139	86
2 cm below skin				112	74
center of corm	58	ND[c]	84	106	64

[a] Corm was from Western Samoa, cultivar Fui. [b] Corms were cvs. 1 and 2 from Finschafen, Papua New Guinea. A decrease of concentration of oxalate and malate was also noted from the growing end (proximal) to the root end (distal) of the stem. No radial gradients of concentration of citrate, malate, and succinate were noted for taro *Xanthosoma*. [c] ND = not detected.

Glutaric acid was chosen as an internal standard because it was well separated from the other organic acids of interest and was not present in the root crops. When added to the samples for analysis, glutaric acid was quantitatively recovered (98–102%). The reproducibility of the method was checked by 10 extractions with dilute H_2SO_4 followed by analyses of the same sample of taro leaves from Fiji. These analyses gave an average oxalate content of 1.61 g/100 g dry weight, with a standard deviation of 0.044 and coefficient of variation 2.8%. The possibility was considered of obtaining an increased value for water-soluble oxalates due to the small solubility of calcium oxalate in water at 25 °C (0.6 mg/100 g solution; Goodenough and Stenger, 1973). This was shown not to be important by an experiment in which the amount of dry sample was increased from 1 to 4 g, without any appreciable change in the experimental result for soluble oxalate.

Gradient of Oxalate Content within Corms. The results in Table II show that there was a considerable decrease in the concentration of total oxalate from the skin into the center of the large corm of giant taro that is typically 1 m long and 15–20 cm in diameter. A similar but smaller gradient of total oxalate was noted in Table II for taro *Xanthosoma*.

The concentration of calcium oxalate, calculated from the difference between total oxalate and soluble oxalate in giant taro, decreased markedly from the skin to the center of the corm. Sunell and Healey (1979) found by light microscopy that the concentration of calcium oxalate raphides decreased from the skin to the center of taro *Colocasia*.

Calcium Oxalate and Acridity. The acridity of giant taro is thought to be concentrated in the outer layers of

Table III. Calcium Oxalate and Organic Acid Content (Milligrams/100 g Fresh Weight) of Taro (*C. esculenta*) Leaves from Fiji[a]

cultivar	total oxalate	sol oxalate	calcium oxalate	total Ca	free Ca	malate	citrate	succinate
Dalo ni Wai[b]	368			182		760	670	
Hawaii[c]	374	72	439	175	38	700	98	239
Vavai Dina[c]	350	132	317	114	15	619	107	317
Toakula[c]	574			297		480	160	
Tausala ni Mumu[c]	278			157		600	110	
Samoa hybrid[d]	483	177	445	157	18	451	188	193
Tausala ni Samoa[d]	532			246		680	170	
Vutikoto[d]	552			204		960	110	
Samoa green[e]	324			159		390	120	
mean	426 (110)	127 (53)	400 (72)	182 (55)	24 (13)	627 (175)	193 (182)	249 (63)

[a] Standard deviations in parentheses. [b] Grown for its edible leaf. [c] Edible leaves. [d] Generally not edible but sometimes eaten. [e] Nonedible leaf.

Table IV. Content of Organic Acid Anions, Calcium Oxalate, and Calcium (Milligrams/100 g Fresh Weight) in Roots and Stems of Tropical Root Crops (Standard Deviation in Parentheses)

root crop and source	name of cultivar	total oxalate	sol oxalate	calcium oxalate	total Ca	free Ca	malate[a]	citrate[a]	succinate[a]
taro *Colocasia*	Samoa green, Samoa hybrid	65 (19)	35 (4)	43	23 (5)	10	107 (29)	102 (31)	168 (51)
Fiji corms, mean of 5 cvs.	Samoa, Toakula, Tausala ni Samoa								
Fiji, suckers,[c] mean of 2 cvs.	Samoa Hybrid, Samoa	60 (1)			14 (2)		ND[b]	ND[b]	ND[b]
taro *Xanthosoma*									
Tonga mean of 3 cvs., 6 samples	Futuna, Maheleuli, Tea	60 (30)	44 (22)	23	6 (1)	0	211 (119)	314 (43)	506 (159)
giant taro *Alocasia*									
western Samoa, mean of 6 cvs., 10 samples	Laufola, Sega, Fui, Toga, Niukini, Uli	38 (18)	17 (9)	31	26 (5)	18	258 (172)	218 (130)	587 (309)
western Samoa, mean of 6 cvs., 10 samples	Niukini, Toga, Sega, Lau Penitala, Faitama, Fui	30 (8)			28 (9)		370 (210)	318 (190)	290
giant swamp taro *Cyrtosperma*									
Fed. States of Micronesia (Ponape), 2 cvs., 3 samples	Nein Alex, Nukuro	319 (77)	45 (39)	399	135 (33)	10	106 (42)	121 (52)	140 (80)
Kiribati, 5 cvs., 12 samples	Ikaraoi Kairoro, Atimainiku, Ikaraoi Ikauraura, Katuta Kairoro, Ikaraoi Natutebubua	300 (218)			219 (156)		170 (170)	50 (60)	450 (670)
elephant foot yam *Amorphophallus*									
Papua New Guinea, 1 cv., 2 samples		288 (134)	25 (6)	382	127	8	513 (68)	256 (81)	339 (140)
sweet potato									
Solomon Islands mean of 6 cvs., 9 samples	Santa Cruz, Three Months, Western, Toni, TIS 2498,[d] Nawaro	94 (45)			29 (8)		101 (30)	140 (101)	ND
Solomon Islands and Fiji, 2 cvs., 8 samples	TIS 2498,[d] TIS 3017[d]	59 (27)	38 (21)	32	30	20	194 (81)	107 (69)	472 (470)
cassava									
Papua New Guinea 2 cvs., 4 samples	Yellow, L12	29	17	17	22	17	438 (354)	258 (142)	343 (335)
Solomon Islands, 3 cvs., 6 samples	Curry Gizo, Betikama, WSH2	48 (14)			19 (6)		206 (95)	388 (160)	ND
yam (*D. alata*)									
Papua New Guinea, 5 cvs.	Takua Kupmi, Kpmora, Takua Yaimbi, Tolai, Yavovi	15 (9)			5.7 (1.5)		123 (55)	127 (61)	ND
Solomon Islands, 4 cvs.	WSH9, GU147, V7, Toki	20 (14)			9.8 (4)		87 (28)	157 (40)	ND
yam (*D. esculenta*)									
Papua New Guinea, 4 cvs.	Mangilmu, Saikidi, Biargu, Glame	17 (10)			6.2 (1.8)		64 (11)	147 (42)	ND
Solomon Islands, 3 cvs., 5 samples	NGP4, GUP4, GUP5	9 (10)			7.3 (4)		102 (8)	99 (34)	ND

[a] Results obtained by extraction with water were the same within experimental error as those obtained by extraction with dilute sulfuric acid; mean values are recorded. [b] ND = not detected. [c] Suckers or cormels grow around the base of the parent corm. [d] TIS 2498 and TIS 3017 are introductions from International Institute of Tropical Agriculture, Nigeria.

the corm and may be largely removed by peeling off a thick layer followed by prolonged boiling (Sakai, 1983). Thus, the large concentration of calcium oxalate in the surface layers would be consistent with the idea that the raphides were a cause of acridity, although Nixon (1987) found that the raphides examined by scanning electron microscopy were not altered by boiling.

The results in Table III show the total oxalate concentrations of nine different cultivars of taro leaves from Fiji, five of which are used commonly as edible leaves, three of which are generally not edible, and one of which is nonedible. It is noted that the content of total oxalate is not significantly different between the cultivars with edible leaves and those with nonedible leaves. Furthermore, the calcium oxalate content of two cultivars with edible leaves was found to be not significantly less than that of one cultivar whose leaves are generally not edible. It is therefore concluded that the acridity present in taro leaves is not solely due to calcium oxalate raphides. There have been many suggestions of possible chemical irritants present, including the glucoside of 3,4-dihydroxybenzaldehyde (Suzuki et al., 1975; Tang and Sakai, 1983). Further studies on the causes of acridity are in progress (Nixon, 1987).

Soluble Oxalate, Calcium Oxalate, and Free Calcium Contents of Root Crops. The results in Table IV show that the content of soluble oxalate (0–50 mg/100 g fresh weight) is small for all root crops. Similar small amounts of soluble oxalates occur widely in vegetables and fruits and do not present a problem (Fassett, 1973). The mean value for water-soluble oxalate of taro *Colocasia* of 32 mg/100 g fresh weight (Wills et al., 1983) agrees well with our value of 36.

The calcium oxalate content in corms of giant swamp taro, elephant foot yam, leaves of taro *Colocasia* (Table III), and the skin of giant taro (Table II) is about 400 mg/100 g fresh weight. This large amount of calcium oxalate is 10 times as great as that present in any of the other root crops. Amounts vary from near zero for yam, to slightly higher values for cassava, taro *Xanthosoma*, sweet potato, giant taro, and taro *Colocasia*.

Many studies have shown that, in man, intake of oxalic acid and soluble oxalates interferes with the assimilation of calcium (Hodgkinson, 1977; Kelsay, 1985). It is also known that, compared with more soluble oxalates, calcium oxalate is less readily absorbed by humans, whereas cows, pigs, and sheep can utilize the calcium present in calcium oxalate due to its breakdown by microorganisms (Brune and Bredehorn, 1961; Hodgkinson, 1977). Atoll dwellers, who use giant swamp taro as a staple and taro leaves as edible greens, would consume a relatively large amount of calcium oxalate (see Tables III and IV). The low incidence of renal stones (which consist predominantly of calcium oxalate; Libert and Franceschi, 1987) among atoll dwellers of the South Pacific (Parkinson, S., personal communication) would be consistent with the poor degree of absorption of calcium oxalate by humans (Hodgkinson, 1977).

The smaller amounts of free calcium, i.e. calcium not present as calcium oxalate, as compared with total calcium present in the root crops are shown in Table IV. The amount available as free calcium appears adequate for most root crops, except for taro *Xanthosoma* where it is zero and for yams where it is small.

The function of calcium oxalate in the plant is not clear (Libert and Franceschi, 1987). In some plants it is metabolized (Smith, 1982), whereas in others it tends to increase in amount during the life of the plant (Fassett, 1973). In giant swamp taro the amounts of total calcium and total oxalate were found to be higher in older plants (Bradbury and Holloway, 1988). The plant may produce needlelike calcium oxalate raphides that may act as a defense mechanism against grazing animals. The defense mechanism may be made much more effective by the presence of a chemical irritant as mentioned above (Nixon, 1987; Bradbury and Holloway, 1988).

Malate, Citrate, and Succinate Contents. The amounts of these organic acid anions were variable from one cultivar to another of the same root crop, from one root crop to another, and in some cases one or more of the organic acid anions was absent. The variability of the results is shown by the large standard deviations in Table IV. This may reflect the active metabolism of these substances in the plant, which could be influenced by biological and environmental factors. The largest mean amounts of 400–600 mg/100 g of malate, citrate, and succinate were obtained with elephant foot yam, cassava, and giant taro, respectively.

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Registry No. Ca, 7440-70-2; calcium oxalate, 563-72-4; oxalic acid, 144-62-7; succinic acid, 110-15-6; malic acid, 6915-15-7; citric acid, 77-92-9.

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Composition, Flavor Extract, Protease, and Glycosidases of Clam Bellies Collected from Clam Processing Plants

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Clam bellies were collected from clam processing plants (A and B) and analyzed for proximate composition, mineral content, and protease and glycosidase activities. The whole clam bellies contained 49.6–52.4% of protein and large amounts of major minerals, phosphorus, calcium, magnesium, potassium, and sodium. The major minerals represented 51% of ash in whole bellies. The flavor extract of clam bellies had unacceptable flavor and was found to be not useful in incorporation of it into human food products. The crude extracts of clam bellies from two plants contained both protease and glycosidases. However, β-glycosidase activity was present in higher levels in the extract of clam bellies than the α-glycosidase activity. Laminarinase, β-1,6-glucosidase, and β-1,4-glucosidase activities were detected in the extracts of clam bellies collected from two clam processing plants.

The clam belly, which constitutes from 7 to 25% of the total meat, is currently underutilized and poses a disposal problem to clam processors (Chen and Zall, 1986a). The solid waste portions (bellies) of the clams are discarded, which include the stomach, liver, and other organs. Chen and Zall (1985, 1986a) have found clam bellies to be good source of different proteases (D-like and B-like acid proteases). They isolated and purified acid proteases from clam bellies and studied some of their characteristics. In a separate study, Chen and Zall (1986b) isolated and characterized clam rennet (which is a crude preparation of cathepsin B-like protease) from clam bellies collected from a clam processing plant and compared the preparation to porcine pepsin and calf rennet for its suitability as a milk coagulant in cheese-making. They reported that clam rennet was more proteolytic and produced a softer curd than the other two coagulants. However, chedder cheese made from clam rennet was inferior to the chedder cheese made from calf rennet.

The crystalline style is part of the clam belly region and contains an assemblage of carbohydrate digestive enzymes that catalyze algal carbohydrate degradation (Shallenberger et al., 1974; Lindley et al., 1976). Four different carbohydrases (laminarinase, amylase, cellulase, alginase) have been characterized from the crystalline style of surf clam bellies (Jacober et al., 1980; Jacober and Rand, 1980). The crystalline style functions by rotating against the gastric shield to grind diatomaceous and algal food while initiating enzyme hydrolysis of carbohydrate polymers (Shallenberger and Herbert, 1974; Lindley and Shallenberger, 1974). Laminarinase was found to be the major carbohydrase in the crystalline style of surf clam. Utilization of clam bellies and its associated parts in the production of commercial enzymes, as a component of livestock or poultry feed and in the production of pet foods, may provide an increased revenue source to clam processors while reducing wastage and meeting minimum waste effluent standards. A study of clam bellies for their proximate composition and mineral content may further enhance their use as a protein ingredient in livestock or poultry feeds and pet foods.

The objective of this study was to analyze clam bellies for proximate composition, mineral content, and protease and glycosidases activities. A flavor extract was also extracted from clam bellies and evaluated for its possible use in human food products.

MATERIALS AND METHODS

Clam Bellies. Flow diagrams of mechanized processes used by three different clam processing plants (plants A–C) in Virginia are shown in Figures 1–3. Plant A processes surf clams (*Spisula solidissima*), while plants B and C process mostly ocean quahogs (*Arctica islandica*). Both surf clams and ocean quahogs receive a preliminary water wash to remove sand and debris from the outside of the shells and are then subjeced to a short heat treatment using either a shucking furnace (50–100 s) or pressurized cooker. The shucking furnace is a large propane furnace reaching temperatures from 625 to 815 °C. A heavy metal chain belt transports the clams through the furnace to

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