Oxalates in Oca (New Zealand Yam) (*Oxalis tuberosa* Mol.)

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Oca (*Oxalis tuberosa* Mol.) or New Zealand yam, in common with other members of this genus, contains oxalate, an antinutritive factor. Twelve South American and two New Zealand cultivars of oca were analyzed for total and soluble oxalate contents of the tubers. The range of total oxalate levels was 92−221 mg/100 g of fresh weight. Levels of soluble and total oxalate extracted from the tubers were not significantly different, suggesting that no calcium oxalate is formed in the tubers. The oxalate concentrations obtained in this study for oca suggest that previously reported values are too low and that oca is a moderately high oxalate-containing food. This is the first report of a tuber crop containing moderate to high levels of soluble oxalates in the tubers and no insoluble oxalates.

Keywords: *Oxalis tuberosa; oca; New Zealand yam; soluble and insoluble oxalates*

INTRODUCTION

Oca (*Oxalis tuberosa* Mol.) is a tuber crop that is cultivated in the Andean region from Venezuela to Argentina (Sperling and King, 1990). Outside South American countries oca has been grown commercially since the 19th century in New Zealand (Hewitt, 1993), where it is known as "yam" (Vietmeyer, 1991; Martin et al., 1997). The tubers of oca have been a staple food item of the Incas (Vietmeyer, 1991). In the Andean region, they are used in soups and stews, steamed, boiled, or baked like potatoes, or served raw as a sweet. In New Zealand, oca is commonly consumed boiled, steamed, or baked, especially as a side dish with roasted meat (King, 1988; Vietmeyer, 1991).

Some Bolivian cultivars of oca, "janko keni", "chime amarillo", and "anko chisme", are bitter, and it is thought that they also contain high levels of oxalates (King, 1988). Sangketkit et al. (1999) also showed that some oca cultivars grown in New Zealand have bitter characteristics which are reduced by cooking.

The oca currently grown in New Zealand has a narrow genetic base, producing only pink tubers. In the Andes, there are cultivars with a range of tuber colors, including red, pink, and yellow, which can be plain or even spotted (Martin et al., 1997). Commercial growers are interested in new cultivars that may increase the appeal of oca to consumers. This could lead to an expansion of demand in both local and foreign markets.

Oxalic acid and its salts, particularly calcium oxalate, can have deleterious effects on human nutrition and health, particularly by decreasing calcium absorption and aiding the formation of kidney stones (Noonan and Savage, 1999). Along with oxalates produced by human metabolism, dietary oxalate is excreted in the urine, but not all oxalates are soluble, and high levels of urinary oxalate can lead to crystallization and stone formation. Soluble oxalate in the diet can bind calcium from other sources in the diet and from the body (Hodgkinson, 1977; Noonan and Savage, 1999).

The levels of oxalates in a variety of foodstuffs analyzed by wet chemistry techniques have been summarized by Zarembski and Hodgkinson (1962), Hodgkinson (1977), and Noonan and Savage (1999). High-performance liquid chromatography (HPLC) and enzymatic methods give more reliable data than earlier titration and wet chemistry techniques (Jordan et al., 1996). Soluble (oxalic acid and soluble salts) and insoluble (predominantly the calcium salt) oxalate can be differentiated by extraction in water to extract soluble oxalates and by extraction in strong acid to extract total oxalates. The difference between acid and water extraction gives the amount of insoluble oxalate in the food (Hodgkinson, 1977; Holloway et al., 1989).

The oxalate content of oca has only previously been measured using wet chemistry techniques by King (1988), who found low levels of total oxalates, ranging from 20.3 to 50.3 mg/100 g of fresh weight (FW). Field trials with many new cultivars recently imported from South America gave an opportunity to retest the oxalate content using modern methods. Fourteen cultivars of oca were measured for soluble and insoluble oxalate content by HPLC, using the extraction and analysis method developed by Holloway et al. (1989).

MATERIALS AND METHODS

Samples. Oca tubers of different flesh and skin colors were collected from local markets in southern Bolivia and northwestern Argentina in 1993 (Table 1) and were brought back to New Zealand, where they were placed in quarantine and tissue cultured until they were free of virus infection. The virus elimination procedure involved the excizing of stem sections and allowing them to grow on a tissue culture medium that contained Ribavirin (Fletcher et al., 1998). The explants were then placed in a growth cabinet set up for heat therapy treatment. The temperature cycled from 35 to 31 °C every 4 h

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Table 1. Description of the Ocas and Location of Collection Sites

cultivar[a]	country of origin	collection site[b]	geographical coordinates[b]	altitude[b] (m)	skin color
L1	New Zealand	Dunedin vegetable market[c]			orange
L2	New Zealand	Christchurch vegetable market[c]			orange
26	Argentina	Escoipie, Salta province	25° 10′ S, 65° 40′ W	2900	dark red
30	Argentina	Escoipie Marae, Salta province	25° 10′ S, 65° 40′ W	2600	white-red
32	Bolivia	Humahuaca, Jujuy province	23° 13′ S, 65° 40′ W	1200	white-red
33	Argentina	Colonzuli, Salta province	23° S, 65° 10′ W	3600	white-red
34	Argentina	Pueblo viejo, Salta province	23° S, 65° 10′ W	3300	cream white
35	Argentina	Pueblo viejo, Salta province	23° S, 65° 10′ W	3300	red
36	Bolivia	Tarija	21° 33′ S, 64° 45′ W	2000	cream white
37	Bolivia	Tarija	21° 33′ S, 64° 45′ W	2000	dark red
38	Bolivia	Tarija	21° 33′ S, 64° 45′ W	2000	red-white
39	Bolivia	Tupiza	21° 27′ S, 65° 45′ W	3400	red
40	Bolivia	Tupiza	21° 27′ S, 65° 45′ W	3400	white-red
41	Bolivia	Tupiza	21° 27′ S, 65° 45′ W	3400	white

[a] Numbers are NZ Institute for Crop & Food Research Ltd. Accession Numbers. [b] Stephan Halloy (personal communication). [c] Generic to New Zealand, originally sourced from South America.

for 2 weeks. Only vigorously growing virus-free plants with no morphological differences from the originally imported plants were released (Fletcher et al., 1998). The oca lines were released from quarantine in June 1994 and subsequently bulked up in field trials. Preliminary yield results have been given by Martin et al. (1997). Twelve South American and two generic New Zealand cultivars, obtained from local produce markets, were analyzed. These were grown in a Templeton loam soil at Crop and Food Research Ltd., Lincoln, Canterbury, New Zealand (172° 29′ E, 43° 39′ S), 11 m above sea level. Experimental plots were one row by 20 m long, in a randomized block design with three replicates. The oca samples were planted November 12, 1996, and harvested on August 12−14, 1997, from a random 3 m section of each plot. The plots were fertilized with Cropmaster (15% N, 10% P, 10% K), 500 kg/ha, immediately prior to planting of the tubers and top dressed with Cropmaster, 200 kg/ha, on January 30, 1997. The total weight of the washed and cleaned tubers >5 cm long (i.e., those that could be sold commercially) from each plot was frozen and stored at −15 °C until analysis could commence.

Extraction of Oxalate. Inedible parts of oca (small blemishes, stems) were removed, but the skin was retained for analysis as this is usually eaten. Individual frozen oca were chopped and mixed, and a 5 g sample was accurately weighed into a 100 mL conical flask. Approximately 50 mL of 2 M HCl (BDH, Poole, U.K.) (pH 0.08), for total oxalate analysis, or 50 mL of distilled water (Barnstead Nanopure II, Dubuque, IA) (pH 7.0), for soluble oxalate, was added to the flask, and the contents were then homogenized using a top-drive homogenizer (Kinematica, Germany). The flasks were capped with Parafilm (Greewich, CT) and placed in a water bath at 80 °C for 20 min and shaken periodically.

Samples were then quantitatively transferred to 100 mL volumetric flasks, cooled, made up to volume with water or acid, and mixed. Forty milliliters of this sample was transferred to 50 mL Falcon tubes (Becton Dickinson, Lane Cove, NSW, Australia) and centrifuged at 1400*g* for 15 min. The supernatant was poured off into storage vials, and a 3−5 mL sample was removed by syringe and filtered through a 0.45 μm cellulose acetate filter (Sartorius, Goettingen, Germany) into a 1 mL HPLC autosample vial (Kimble, Vineland, NJ). Replicates were extracted and measured separately. Each sample of oca was analyzed in duplicate.

Preparation of Standards. Standards of oxalic acid (BDH) and calcium oxalate (Aldrich, Milwaukee, WI) were made up in the following concentrations: 1.0, 5.0, 10.0, 20.0, 40.0, 50.0, and 100.0 mg/100 mL, made up in either 2 M HCl (AR, BDH) or distilled (Barnstead Nanopure II) water. Standards were made up to volume in 100 mL volumetric flasks and then filtered through a 0.45 μm cellulose acetate filter (Sartorius) into a 1 mL HPLC autosample vial (Kimble).

Recoveries. Ten milligrams of oxalic acid (BDH) was added to acid and water extracts of one cultivar (No. 35) prior to homogenization and then treated in the same as the other samples in each run. Calcium oxalate (Aldrich) was added to water and acid extracts in the same manner to determine whether calcium oxalate was being measured as soluble oxalate in the water extract.

Analysis of Oxalates. HPLC of extracted oxalic acid was carried out on a 300 × 7.8 mm ion exclusion column (HPX-87H, Bio-Rad, Hercules, CA). Mobile phase was 0.0125 M sulfuric acid (Hypersolv, BDH). The equipment consisted of a Waters LC Module I HPLC (Waters, Milford, MA) with a UV detector set at 210 nm. Five microliter samples were injected in duplicate onto the column and eluted at a flow rate of 0.6 mL/min. The oxalic acid peak eluted at 7.00 min. Data were analyzed using Millennium Chromatography Manager ver. 2.15 software. Results were calculated as milligrams per 100 g of fresh weight, taking water of hydration into account (BDH oxalic acid contains 71.4% oxalic acid).

Enzyme Analysis of Oxalate. To confirm the validity of the HPLC results and to compare the effectiveness of HPLC and enzyme assay, an enzyme kit for oxalate determination (Boehringer Mannheim, 1997) was used to analyze two samples previously analyzed by HPLC. Extraction was the same as for the HPLC extraction method, except that concentrated HCl was added until the pH of the extract reached pH 2−3. The same sample was measured by HPLC, and recoveries were calculated in the manner described above.

Calculation of Soluble and Insoluble Oxalate. The pH of the acid and water extractions were different (water, pH 7.00; 2 M HCl, pH 0.08). Calcium oxalate, the main insoluble oxalate found in plants, has solubilities in water of 0.0086 g/L at 25 °C and 0.0145 g/L at 95 °C, but it is soluble in acid (Hodgkinson, 1977). A hot water extract would not be able to dissolve the insoluble oxalate component, whereas 2 M HCl extraction would solubilize insoluble and soluble oxalates. The difference between acid (total) and water (soluble) extractions is assumed to be insoluble oxalate (Holloway et al., 1989).

RESULTS AND DISCUSSION

Yields. The mean yields of edible oca ranged from 0.68 to 13.3 t/ha (Table 2). Both of the generic New Zealand ocas gave good yields in this field trial. Nearly all of the recently imported cultivars, except for cultivar 38, gave yields that would be uneconomic for commercial production. The mean weight of the tubers >5 cm in length ranged from 16.5 to 37.4 g/tuber. The two generic oca tubers had very similar tuber sizes.

Recoveries. Recoveries for acid extractions had a mean of 99 ± 1.7%, indicating that the method used here is effective for the analysis of total oxalate. The water extractions were more variable but are still acceptable, with a mean percent recovery of 89 ± 3.6. It is possible that the lower recovery of the water extract may be due to some oxalic acid binding to fiber in the sample during the extraction process. However, Ross

Table 2. Yield, Tuber Weight, and Levels of Oxalate in the Tubers

cultivar[a]	yield (t/ha, mean ± SE)	tuber wt (g, mean ± SE)	dry matter (%)	soluble oxalate (mg/100 g of FW ± SE)	total oxalate (mg/100 g of FW ± SE)
L1	10.04 ± 1.22	24.05 ± 3.90	17.40	171 ± 3.1	162 ± 3.7
L2	9.04 ± 0.96	24.43 ± 2.08	12.82	137 ± 5.5	128 ± 3.7
26	3.83 ± 0.49	21.89 ± 4.28	22.26	92 ± 8.4	93 ± 6.6
30	0.68 ± 0.16	16.54 ± 5.22	15.76	203 ± 16.0	203 ± 15.4
32	4.36 ± 0.25	26.96 ± 3.89	18.24	213 ± 9.4	222 ± 12.9
33	5.35 ± 1.01	26.56 ± 2.00	18.99	156 ± 3.5	154 ± 5.4
34	3.86 ± 1.21	21.88 ± 4.28	14.73	113 ± 4.8	113 ± 9.5
35	4.29 ± 0.62	25.39 ± 5.95	15.58	201 ± 12.2	199 ± 12.1
36	4.72 ± 1.12	31.10 ± 3.99	16.58	129 ± 5.2	126 ± 4.5
37	3.81 ± 0.53	25.77 ± 9.26	14.32	180 ± 8.7	190 ± 12.4
38	13.34 ± 1.52	37.39 ± 2.85	15.65	203 ± 1.6	186 ± 15.1
39	3.90 ± 0.95	21.43 ± 3.80	20.51	147 ± 2.6	141 ± 6.3
40	5.53 ± 0.56	20.88 ± 3.12	14.65	153 ± 13.1	153 ± 20.8
41	1.38 ± 0.22	21.38 ± 4.16	15.27	134 ± 9.5	121 ± 17.3
LSD ($p < 0.001$)	2.44	3.80		44.85	56.94

(1998) showed that an insignificant (3 mg of oxalate/5 g of oca tuber extracted) amount of oxalate could become bound to oxalate-free fiber extracted from oca tubers using the neutral detergent fiber method of van Soest and Wine (1967). Holmes et al. (1995) showed that re-extracting the residual fiber of Bran Flakes breakfast cereal after oxalate analysis yielded no further oxalate.

Levels of Oxalates in Oca. The levels of oxalates in oca varied greatly between cultivars and ranged from 92 and 113 mg/100 g of FW for cultivars 26 and 34 to 201−213 mg/100 g of FW for cultivars 30, 32, 35, and 38 (Table 2). None of the cultivars have excessively high levels of oxalate compared to other commonly consumed foods such as spinach, which can have up to nearly 10 times as much oxalate (320−1260 mg/100 g of FW) (Noonan and Savage, 1999). Levels are higher than for many other foodstuffs consumed in a traditional English diet (Zarembski and Hodgkinson, 1962), which is unlikely to be too different from the traditional New Zealand diet. The only exceptions to this are rhubarb and spinach, which have very high levels of oxalate (Noonan and Savage, 1999).

The level of oxalates found in all cultivars of oca in this study are higher than that found in the one sample of New Zealand oca measured by King (1988) using the titration method (Association of Official Analytical Chemists, 1984), which gave a mean value of 52 mg/100 g of FW. The oca samples measured by King (1988) were fixed in ethanol (percent not stated) before being taken to the United States for analysis. This could have caused the loss of some oxalate into the fixing solution, which was not analyzed. Carvalho et al. (1995) used 12% ethanol to extract oxalate for analysis, so it is quite possible that some oxalate had leached into the ethanol solution in King's study. Oxalate is found in highest concentrations in the skin of tropical root crops (Holloway et al., 1989), which would be most vulnerable to losing oxalate to the surrounding ethanol if oca follow the same pattern. Even the lowest amount of oxalate in any cultivar in this study, 92 mg/100 g of FW, is 40 mg greater than reported by King (1988). Holmes et al. (1995) and Jordan et al. (1996) have already identified inaccuracies in the AOAC method (Association of Official Analytical Chemists, 1984). Other possibilities to explain the differences in oxalate level between this study and King's are the location and the treatment of the oca with fertilizer and irrigation, which could produce tuber oxalate levels significantly different from those of the oca samples analyzed by King (1988). In this experiment all the ocas were given the same irrigation and fertilizer treatments and the levels applied were modest (Martin et al., 1999).

The acid extract gave the same values ($p < 0.05$) as the water extract (Table 1), which indicates that there is no insoluble oxalate in oca (Bradbury and Holloway, 1988; Holloway et al., 1989). This is the first time that an analysis of insoluble oxalates has been carried out on a tuber crop. Potato tubers have been shown to contain low levels of soluble oxalates (Noonan and Savage, 1999; Hodgkinson, 1977), but no analysis of insoluble oxalate has been undertaken so far.

Enzymic analysis of two cultivars of oca yielded a mean value of 113.30 mg of oxalate/100 g of FW for cultivar 26 and 188.83 mg of oxalate/100 g of FW for cultivar 32. Both values are a little different from those obtained using 2 M HCl at 80 °C (Table 2) and HPLC analysis. Jordan et al. (1996) has noted that the enzyme assay is inaccurate outside its detection limit, and Boehringer Mannheim states that their kit method is subject to a number of interfering compounds such as L-ascorbate and lipids.

Oca does not appear to contain any calcium oxalate even though it contains a modest amount of calcium (Milligan, 1988; Ross, 1998). This is in contrast to tropical tuber crops analyzed by Holloway et al. (1989), which contain high levels of calcium oxalate. Oca, however, contains high levels of soluble oxalate compared with other high-oxalate foods. Soluble oxalate is more readily absorbed from the gastrointestinal tract than is insoluble oxalate and may be able to bind calcium from other sources in the diet, making calcium from the diet unavailable (Hodgkinson, 1977).

The lack of calcium oxalate may also have important implications in pest resistance (Bradbury and Hammer, 1990) and on sensory qualities (Sangketkit et al., 1999) of oca tubers. Calcium oxalate raphides are thought to be responsible for sharpness of taste and acidity in some foods, such as kiwifruit (Broschat and Latham, 1994). Oca with higher levels of soluble oxalate may have a tangier flavor than low-oxalate varieties (Sangketkit et al., 1999). This may make oxalate concentration an important factor in the selection of oca cultivars for commercial production.

The level of oxalate in these tubers is not a major concern as ~1 kg of raw high-oxalate oca would need to be consumed at one time to ingest 2 g of oxalate, which is thought to be a fatal dose for humans (Libert and Fransceshi, 1987). However, cooking has been shown to reduce the oxalate level of foods. Boiling has been shown to be most effective in reducing the oxalate

content of tropical legume seeds, by 19.5−39.3%, whereas roasting and autoclaving were less effective as losses were only 5.2−8.2 and 1.9−5.7%, respectively (Apata and Ologhobo, 1997). Savage et al. (1999) also noted that cooking reduced soluble oxalate levels in common oxalate-containing vegetables.

Oxalate bioavailability is an important factor in the determination of whether a food is a high risk for people with hyperoxaluria, which is a risk factor in the formation of kidney stones (Brinkley et al., 1990). It is normal to restrict the oxalate content in the diet of such people, but Brinkley et al. (1990) suggest that only foods with high levels of bioavailable oxalate (>20 mg increase in urinary oxalate/ load), such as spinach (29.3 mg/load), are major risk factors. However, the proportion of oxalate that is soluble is also an important factor, as soluble oxalate is more bioavailable than insoluble oxalate (Brinkley et al., 1990). Thus, the consumption of more than modest amounts of oca should be considered carefully for people suffering from hyperoxaluria.

Conclusions. Oca grown in temperate conditions in New Zealand contain moderately high levels of oxalates but not as high as other commonly consumed foods, such as spinach and rhubarb. This is the first report of a tuber crop containing only soluble oxalates in its tuber.

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