# Oxalate Content of some Leafy Green Vegetables and its Relation to Oxaluria and Calcium Utilization

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Since the work of Fincke & Sherman (1935) on the availability of the calcium of spinach, it is generally agreed that the presence of oxalates in the food impairs the absorption of calcium. Confirmatory findings on dog, man and rat have been reported by McClugage & Mendel (1918), Fincke & Garrison (1936), and Fairbanks & Mitchell (1938). Fairbanks & Mitchell (1938) state that the addition of sodium oxalate to diets of skimmed milk powder depresses the calcium utilization by 24-38 %. The interference of phytic acid with the utilization of calcium of cereals has also been recognized for some time, and it has found practical application in England where 14 oz. (370 g.) of calcium carbonate are added to every 280 lb. (129 kg.) of 85 % flour to neutralize the effect of phytic acid in wheat (Peters, 1942).

It is obvious from these facts that calcium determinations on foodstuffs are of little significance from the nutritional point of view, unless taken in conjunction with the oxalic or phytic acid content or both. Thus Fairbanks & Mitchell (1938) found that the calcium of spinach (Ca, 0.086%; oxalic acid, 0.29-0.69%) was hardly used by the rat.

Closely associated with this problem is the incidence of oxaluria following the ingestion of oxalaterich foods. Though this fact has been known for a long time, reliable and comprehensive data on the oxalate content of foods have been lacking until recently when Kohman (1939) published figures for the oxalic acid content of a large number of foodstuffs. In view of the low calcium in the dietary of the average Cingalese (Nicholls & Nimalasuriya, 1939), the availability of calcium in relation to the oxalic acid content of the diet is a problem of some importance to tropical nutrition. We have therefore estimated the oxalic acid of some of the indigenous foodstuffs, and have confined ourselves to leafy green vegetables which meet most of the mineral and vitamin requirements of the Cingalese.

## **METHODS**

The stalks and stems were discarded and only the leaves were used, in order to obtain reproducible results. They were spread in thin layers on stainless steel wire mesh for 24 hr. at room temperature ( $28^{\circ}$ ) and later dried in an air oven at  $60^{\circ}$ . The dried material was finely powdered.

Calcium. Ca determinations on this material were carried out according to the method described in the *Technical Communication* No. 9 of the Imperial Bureau of Animal Nurition (1937).

Oxalic acid. The method used was that of Arbenz as described by Nelson & Mottern (1931), with certain modifications. Preliminary experiments showed that (1) oxalic acid was not destroyed during acid digestion of the powdered material, (2) during concentration of the acid digest there was considerable loss of oxalic acid even at 60° (this loss is attributed to decomposition of the oxalic acid formed on the sides of the evaporating basin), and (3) 20–30 hr. extraction with ether was necessary for the complete removal of oxalic acid when a Quickfit continuous-flow liquid extractor was used. Based on these observations, the following procedure was adopted.

About 20 g. of the powdered material were weighed out and digested in a round-bottomed flask with 200 ml. 1·5 N-(approx.) HCl for 12 hr. on a sand-bath under a reflux condenser. The contents were then filtered through a Buchner funnel, the residue was washed several times with hot water, and the filtrate and washings were made to 375 ml. Samples (70 ml.) of the solution were then subjected to a 24 hr. extraction in an air-bath maintained at 60–70°. A mixture of 200 ml. ether (A.R.) and 50 ml. distilled water was introduced into the flask at the start of the ether extraction, because preliminary experiments showed that addition of water prevented decomposition of oxalic acid, which as it gets extracted passes readily into the aqueous phase.

At the end of the extraction more water was added, the ether was distilled off and the aqueous solution was filtered. The filtrate was made slightly alkaline with NH<sub>3</sub>, then slightly acid with acetic acid, heated on a water-bath and the oxalic acid precipitated as calcium oxalate by addition of a saturated solution of CaCl<sub>2</sub>. Whenever the precipitate was coloured, reprecipitation was carried out until the material was almost white. The oxalate was then estimated in the usual way with 0·1 n·KMnO<sub>4</sub>. The extractions and precipitations were always carried out in duplicate and the average values taken.

No special advantage was observed in the method described by Kohman (1939) over our modified Arbenz method. Further, all his values have been expressed as a percentage of the wet material, and the moisture content is a variable factor.

The method was slightly modified for oxalate estimation of tea beverage, which was prepared according to the instructions issued by the Tea Propaganda Board, Ceylon. To 50 ml. of the water extract were added about 25 ml.  $1\cdot5$  n-(approx.) HCl. The solution was heated on a waterbath for 30 min., cooled, and the extraction and precipitation carried out as before.

### RESULTS

The vegetables were analyzed as described above and the results are presented in Table 1.

in these vegetables. It is suggested that Gasniviti and Araikeerai, in spite of their popularity in the dietary in Ceylon, should be particularly avoided.

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As already stated, the calcium intake of the

Table 1. Calcium and oxalic acid contents of some Cingalese vegetables

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Name		Mois-	Calcium		Oxalic acid (anhyd.)		
		ture	Fresh	Dried	Fresh	Dried	Available Ca
Botanical	Cingalese	(%)	(%)	(%)	(%)	(%)	(% of total)
I pomoea aquatica	Kankun	85.5	0.132	0.910	0.321	2.214	Oxalic acid in excess
Talinum speciosa	Gasniviti	94.5	0.152	2.760	0.700	12.720	Oxalic acid in excess
Amaranthus polygonoides L.	Araikeerai	85.9	0.595	4.220	1.586	11.250	Oxalic acid in excess
Alternanthera sessilis	Mukunuvenna	$86 \cdot 2$	0.226	1.640	0.942	6.822	Oxalic acid in excess
Sesbania grandiflora	Katurumurunka	$82 \cdot 3$	0.302	1.700	0.149	0.839	<b>78</b> ·1
Dentella asiatica	Gotukola	$84 \cdot 3$	0.206	1.310	0.162	1.034	64.9
A $mar$ $anthus$ $g$ $ang$ $e$ ticus	Gusthampola	$85 \cdot 2$	0.517	3.490	0.891	6.022	23.3
Cassia tora	Thorakola	_	_	$2 \cdot 190$		0.263	94.7
Tea leaves		_	.—	0.466		1.420	
Cup of tea		_		(	)·055 (g./4	00 ml. extr	act) —

#### DISCUSSION

As seen in Table 1, the determinations of both the calcium and oxalic acid contents of a foodstuff are of some significance in assessing its nutritive value. The figures in the last column of the table were obtained by subtracting from the total calcium the amount of this mineral required to precipitate all the oxalic acid in the vegetable as calcium oxalate, and expressing this difference as a percentage of the total calcium. If it is assumed that calcium oxalate is not absorbed in the intestine (Adolph & Liang, 1942; Fairbanks & Mitchell, 1938), then this percentage is more or less an index of the availability of the calcium in the foodstuff. On this assumption, nearly 95% of the calcium in Thorakola is available for absorption. Though Gusthampola has a higher percentage of total calcium, only 23 % of it is available. On the other hand, in Kankun, Gasniviti, Araikeerai and Mukunuvenna, oxalic acid is in excess of calcium, all of which is therefore not available. Ingestion of the latter vegetables would lead to oxaluria, whereas the oxalic acid contents of the other four vegetables are of no significance in oxaluria because of the preponderance of calcium average Cingalese or Indian is much below the standard recommended by the National Research Council (1943). Aykroyd (1941) states that the daily calcium intake of the adult Indian is less than 0.2 g., compared with 0.8 g. recommended by the National Research Council. Consumption of oxalaterich foods would further aggravate the already existing deficiency in calcium. From a practical point of view it would be better to avoid oxalaterich foods than to take measures to neutralize the effect of oxalic acid, especially when other sources of green vegetables are available.

Tea has been included in our investigation because of its popularity as a beverage among all classes of Cingalese. A cup of tea brewed in the usual way contains about 55 mg. oxalic acid, an amount not likely to interfere seriously with the absorption of the calcium of the milk added to tea.

#### SUMMARY

- 1. The calcium and oxalic acid contents of a number of vegetables are reported.
- 2. A modification of the Arbenz method of estimating oxalic acid is described.
- 3. The relation of oxalic acid to calcium availability and oxaluria is discussed.

#### REFERENCES

Adolph, W. H. & Liang, C. C. (1942). J. biol. Chem. 146, 497.

Aykroyd, W. R. (1941). The Nutritive Value of Indian Foods and the Planning of Satisfactory Diets. Health Bulletin no. 23. Calcutta: Government of India Press.

Fairbanks, B. W. & Mitchell, H. H. (1938). J. Nutrit. 16, 79.

Fincke, M. L. & Garrison (1936). J. Home Econ. Proc. 28, 572

Fincke, M. L. & Sherman, H. C. (1935). J. biol. Chem. 110, 421.

Imperial Bureau of Animal Nutrition (1937). Technical Communication, no. 9.

Kohman, E. F. (1939). J. Nutrit. 18, 233.

McClugage, H. B. & Mendel, L. B. (1918). J. biol. Chem. 35, 353.

National Research Council (1943). Recommended Dietary Allowances. Washington: National Research Council.

Nelson, E. K. & Mottern, H. H. (1931). J. Amer. chem. Soc. 53, 1909.

Nicholls, L. & Nimalasuriya, A. (1939). J. Nutrit. 18, 563. Peters, R. A. (1942). Nature, Lond., 150, 125.