Br. J. Nutr. (1978), 40, 591

Effect of water-soluble oxalates in *Amaranthus* spp. leaves on the absorption of milk calcium

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(*Received* 10 *May* 1978 – *Accepted* 19 *July* 1978)

1. *Amaranthus* spp. leaves contain high amounts of oxalates which affect the calcium absorption. This study was done to determine whether removal of the water-soluble oxalates from the leaves by cooking would reduce this deleterious effect.

2. Experimental work done with two types of basal diets on six adult male subjects has shown that the milk Ca absorption was low when leaves cooked without draining away the water were included in the diet. However when the soluble oxalates were removed by throwing away the water after cooking the leaves, the absorption of milk Ca was unaffected.

It is well established from the studies carried out by many workers on animals that oxalates present in foods interfere with the assimilation of calcium (Kohman, 1939; Lovelace *et al.* 1950; Oke, 1969). The work done by us previously on human subjects (Pingle & Ramasastri, 1978) has shown the Ca present in *Amaranthus* spp. leaves is not available for absorption and also that consumption of these leaves together with milk renders milk Ca less available for absorption. This was probably due to the high oxalate content of the leaves. It is known that oxalates occur in foods in water-soluble and water-insoluble forms, and it has been stated by some workers (Srivastava & Krishnan, 1959; Singh *et al.* 1969) that the water-soluble oxalates can render Ca unavailable to the body. There is however no experimental evidence, especially on human subjects, for this statement. Green leaves are an important source of nutrients, for example β-carotene and iron, and hence their consumption is advisable. Since most of the leaves which are commonly consumed in significant amounts are rich in oxalates, it appeared worthwhile to determine whether by removal of water-soluble oxalates the deleterious effect of the leaves on absorption of Ca from milk could be overcome. The results of such a study are presented in this communication.

MATERIALS AND METHODS

Subjects and experimental procedures

Healthy male subjects aged 20–35 years and belonging to the scientific staff of the National Institute of Nutrition, Hyderabad, were selected for the study. In each of Expts 1 and 2, six subjects were selected and were given meals of the following four diets in turn. Diet 1 was a basal diet and diet 2 was the same basal diet with milk added to it. Diets 3 and 4 consisted of diet 2 with the addition of 100 g *Amaranthus* spp. leaves; the only difference between the two diets was that the *Amaranthus* spp. leaves were given with and without the cooking water in diets 3 and 4 respectively.

The basal diet used in Expt 1 consisted of sago (*Manihot esculenta*) (80 g) as described in the previous communication (Pingle & Ramasastri, 1978) and in Expt 2 consisted of a meal containing 150 g rice and 26 g pulse (pigeon pea, *Cajanus cajan*) to each of the subjects to simulate a usual south Indian meal. The rice was cooked in the usual way and the pulse was cooked like soup and made into a south Indian preparation called sambar

Table 1. *Effect of cooking* Amaranthus *spp. leaves on the content (/100 g) of some of the nutrients*

Nutrient analysed	No. of samples	Raw leaf Range	Raw leaf Mean	Cooked leaf (water drained away) Range	Cooked leaf (water drained away) Mean	Loss during cooking (%) Range	Loss during cooking (%) Mean
Oxalic acid (anhydrous) (mg)							
Water-soluble	3	378–527	446	26–32	30	91–95	93
Water-insoluble†	3	850–1058	956	958–1076	1009	—	—
Riboflavin (μg)	2	280–433	356	40–55	47	86–87	87
Folic acid (μg)	2	253–275	264	31–46	38	83–88	86
Ascorbic acid (mg)	2	80–120	100	5–9	6	93–94	94
β-carotene (mg)	2	3·10–7·28	5·19	3·55–7·96	5·75	—	—
Calcium (mg)	5	285–626	455	298–723	473	—	—
Iron (mg)	5	5·4–19·5	9·5	2·8–10·5	5·2	35–50	45
Phosphorus (mg)	3	58–107	89	15–37	26	64–76	70

† Calculated by subtracting the water-soluble oxalic acid content from the 'total' oxalic acid content.

by adding small amounts of spices, tomatoes and onions. The milk given to the subjects contained approximately 600 mg Ca.

After the experimental diet was given to the subjects, urine was collected for a period of 6 h according to the method of Walker *et al.* (1975) as described in a previous communication (Pingle & Ramasastri, 1978).

ANALYTICAL METHODS

To determine the effect of cooking on the loss of oxalates and some of the important nutrients, two or more samples of *Amaranthus* spp. leaves were collected randomly and analysed. These nutrients were analysed both in the raw state and in leaves which had been cooked for 15 min in fifteen times its weight of water and the water thrown away. The oxalic acid (total and water soluble) content of the leaves were analysed by the method of Baker (1952). Riboflavin and folic acid were estimated by the microbiological method described by Srinivasa Rao & Ramasastri (1969) and Lakshmiah & Ramasastri (1975) respectively. Ascorbic acid was estimated by the visual titration method described by the Association of Vitamin Chemists (1966) and the method used for β-carotene estimation was essentially that given by Friend & Nakayama (1959). Fe, Ca and phosphorus contents were estimated by the method described by the Association of Official Agricultural Chemists (1965).

The amounts of Ca in the urine and milk were estimated by the complexometric method described in the previous communication (Pingle & Ramasastri, 1978).

RESULTS AND DISCUSSION

The result of cooking *Amaranthus* spp. leaves and throwing away the water on the oxalic acid content as well as the content of some of the other important nutrients are shown in Table 1. There was a 90% reduction in the total oxalate content and this was mainly due to the loss of the water-soluble oxalates originally present in the raw leaves. There was, however, no loss of the insoluble oxalates.

The loss of water soluble vitamins like riboflavin, folic acid and ascorbic acid due to this cooking method was considerable, ranging from 86 to 94%. However, there was no loss in β-carotene content of the green leaves.

Table 2. *Expts* 1 *and* 2.† *Urinary excretion of calcium (mg Ca/6 h) on a diet containing milk and* Amaranthus *spp. leaves given with and without the cooking water*

(Mean values with their standard errors for six subjects in Expts 1 and 2)

				Urinary Ca excretion			
Expt‡		Basal diet	Milk diet	Milk+ *Amaranthus* with cooking water	Milk+ *Amaranthus* without cooking water	Milk− (milk+ *Amaranthus* with cooking water)	Milk− (Milk+ *Amaranthus* without cooking water)
1	Mean±SE	58·3±9·97	104·65±11·53	79·95±13·15	102·2±8·23	24·7±5·07**	2·45±6·36
	Median	48·75	106·90	90·50	91·80	24·70	0·05 NS
2	Mean±SE	61·38±5·00	108·45±5·71	80·57±5·406	96·65±4·88	27·88±3·61***	−9·4±3·724
	Median	69·25	111·95	76·5	86·0	22·25	−4·75 NS

NS, not significant.
Values were statistically significantly different: $^{**}P < 0.01$, $^{***}P < 0.001$.
† For details of experimental procedures, see p. 591.
‡ Expt 1 with basal diet as sago and Expt 2 with basal diet consisting of rice and pulse (pigeon pea (*Cajanus cajan*)).

Except for Ca, minerals like Fe and P were lost to a significant extent during this cooking process, the loss of Fe being approximately 45% and that of P being 70%.

The urinary Ca excretion over a 6 h period in the six subjects in Expts 1 and 2 are given in Table 2. The results confirmed the previous findings (Pingle & Ramasastri, 1978) that although the absorption of Ca from milk was good, it was reduced when given together with *Amaranthus* spp. leaves cooked in the usual way without draining away the water. However, when milk was taken along with *Amaranthus* spp. leaves which were cooked and water drained away, there was no significant difference in urinary Ca excretion as compared to the excretion when milk alone was given to the subjects.

It can be seen from the results obtained in the present study that the deleterious effect of consumption of *Amaranthus* spp. leaves on milk Ca absorption was removed during the process of cooking the green leaves and throwing away the water and this is probably due to the removal of water soluble oxalates from the leaves during the process of cooking.

During the process of removing the water soluble oxalates, other important nutrients such as vitamins like folic acid, ascorbic acid and riboflavin and minerals like Fe were also lost to a considerable extent. There was however no loss of β-carotene which is a fat soluble vitamin. Ca also was not lost by this method of cooking and this was probably because all of it is in the insoluble form as the oxalate.

In growth studies on rats, Rau & Murthy (1942) have also shown that the Ca from *Amaranthus gangeticus* is unavailable and that the leaves render part of the skim-milk Ca also unavailable. They have shown that these effects are reduced by inclusion of rice in the diet. The present study does not support the finding that rice improves the availability of Ca of *Amaranthus* spp. leaves.

It is well known that leafy vegetables are rich sources of a number of nutrients such as vitamin A, riboflavin, folic acid, ascorbic acid and Fe. People are therefore usually encouraged to consume significant amounts of these leafy vegetables. Our present work suggests that if the vegetables are rich in oxalates, the water soluble forms have to be removed so that their deleterious effects such as rendering Ca from other sources unavailable can be overcome. But it should be noted that during this process significant amounts of some of the important nutrients are lost.

The authors thank Mr E. P. Balakrishnan for his technical assistance in the microbiological assays and are grateful to Dr C. R. Rao, FRS, Jawaharlal Nehru Professor, Indian Statistical Institute, New Delhi, for critically reviewing the statistical analysis of the results. They thank Dr S. G. Srikantia, Director, National Institute of Nutrition for the useful discussion and help rendered and thank the experimental subjects for their co-operation.

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Printed in Great Britain