

Original article

Forms and contents of oxalate and calcium in some vegetables in Ethiopia

Kasaye Jiru and Kelbessa Urga¹

Abstract: Contents and forms of oxalate and calcium in some vegetables of Ethiopia were determined. On the average, the total oxalate content of spinach, swiss-chard and beet root were 9794,7554 and 222 mg/100g on dry weight basis, respectively. The other vegetables studied however, contained low level of oxalate. Two fractions of oxalate were isolated- a fraction soluble in boiling water and an associated (nsoluble residue which was predominantly in the form of calcium oxalate. The water-soluble oxalates of vegetables studied account for over 50% of the total oxalate. Most of the calcium in the vegetables was in the form .of calcium oxalate whirh is unlikely to be available to the body. Since the intake of vegetables cannot be discouraged, it is worthwhile to improve the nutritional value of these vegetables by reducing the water-soluble oxalates as it accounts for over 50% of the total oxalate content. [Ethiop. J. Health Dev. 1995;9{1):13-18]

Introduction

The majority of the population in Ethiopia, obtain their nutritional requirements from plant foods such as cereals, legumes and vegetables. The intake of foods of animal origin such as milk products, meat, eggs and fish is small (1).

Vegetables, particularly green leafy vegetables, are inexpensive and rich sources of several nutrients such as calcium, iron, carotene, ascorbic acid and in some cases group vitamins (2, 3). Protein concentrates from leaves (novel foods) are good sources of protein and are included in balanced diets especially for vulnerable segments of the population. Cultivation of green leafy vegetables in kitchen gardens was also encouraged in order to richly supplement the diets of pregnant and lactating mothers and children (4, 5). Fruits and vegetables are also crucial in efforts to prevent micronutrient deficiencies (6). However, the main constraint to their nutritional exploitation is the presence of some antinutritional and toxic factors such as nitrate and oxalate (7, 8, 9).

Oxalic acid is a dicarboxylic acid, and its salts are considered to be antinutrients as well as toxins. They can render some mineral nutrients unavailable by binding them to form insoluble salts which are not absorbed by the intestine. In addition, ingestion of large amounts of oxalic acid can be toxic (3, 10). The main interest in oxalate has been its complex formation with calcium to form water insoluble calcium oxalate within both plants and animals, which presumably precludes the utilization of calcium by the body. However, water-soluble oxalates and free oxalate can also combine with calcium from other foods in the intestinal tract and further reduce calcium availability (2, 10).

Fruits, green leafy vegetables, and tubers traditionally cultivated in Ethiopia have been reviewed by Zemedet et al (11). In most areas of Ethiopia, the cultivation and intake of green leafy vegetables is low except during rainy seasons when many types of edible leaves and roots, wild and cultivated are consumed. The low intake of green leafy vegetables in most areas of Ethiopia has resulted in

increased prevalence of vitamin A deficiency (12). The limited consumption of green leafy vegetables may be due to lack of appreciation of their nutritional value, avoidance because of beliefs and taboos, non-availability in markets and high price. In ensete (*Ensete ventricosum*) cropping areas, kale is consumed along with ensete foods thus making part of the daily diet. Kale which contains more protein than ensete, is also one of the main sources of protein in these areas (13).

¹From the Ethiopian Nutrition Institute, P.O.Box 5654, Addis Ababa, Ethiopia

Vegetables are believed to be consumed mainly in some larger towns where one can find a variety of them at vegetable stands and shops all year round. Even in these towns, vegetables are consumed mainly during periods of fasting (14).

Several studies on oxalate content of various foods have been carried out elsewhere (8, 9, 15). However, the oxalate content of the vegetables cultivated in Ethiopia is not known. The present study was, therefore, undertaken with the objective of identifying the forms and to determine the content of oxalate in some vegetables collected from markets in Addis Ababa. The content of minerals such as calcium, phosphorus, and iron in the vegetables was also studied.

Methods

Sample collection Vegetable samples for the study include potato (*Solanum tuberosum*), beet root (*Beta vulgaris*), sweet potato (*Ipomoea batatas*), carrot (*Daucus carota*), cabbage (*Brassica oleracea capitata*), kale (*Brassica carinata*), lettuce (*Lactuca sativa*), swiss-chard (*Beta vulgaris cicla*) and spinach (*Spinacia oleracea*). The selection of the vegetables for the study was merely based on their availability throughout the study period. The vegetable samples were collected from fourteen sites in Addis Ababa of which nine were open markets and five from State shops. These sites are reputed for the sale of vegetables throughout the year. Every effort was made to obtain freshly harvested samples which were then placed in polyethylene bags to minimize contamination and stored from 1 to 15 days in a cool room maintained at 4 °C before analysis.

Sample preparation. All the vegetable samples were thoroughly washed and rinsed in deionized water to remove all soil, drained and shredded using an Osterizer (Oster, USA). Samples of freshly shredded materials were dried at 60 °C in an air circulation oven to constant weight. The dried samples were ground to pass through Imm mesh screen using a Cyclotec sample mill (Cyclotec, Tecator-AB, Sweden) and were used in all analyses.

Analytical methods. The moisture content of the fresh vegetable samples was calculated from the loss in weight during drying at 100 °C for 12 hrs in a drying oven (16). Calcium and iron contents were estimated by the method described by the AOAC (16). Total phosphorus was determined according to the method of Fiske and Subbarow (17). The oxalates in vegetable samples were separated into two fractions using the following

procedure: Two grams of finely ground plant material was extracted with 100 ml of boiling deionized water for 30 min, filtered and adjusted to 200 ml. The hot water extract residue was

further extracted with 150 ml of boiling IM HCl for 30 min, adjusted to 200 ml, and filtered. The oxalate concentration in the two fractions was determined as outlined in AOAC (16) with potassium permanganate titration. The content of calcium oxalate and free calcium (mg/100g dry weight) was calculated according to Bradbury and Holloway (18). All the analyses were carried out in triplicate and the results calculated and expressed on dry weight basis.

Results

The dry matter content of vegetables is presented in Table I. The dry matter content was high in sweet potato and potato indicating the lowest moisture content in these vegetables. The dry matter content was, however, quite low in lettuce and swiss-chard, 6.4% and 8.3%, respectively. Consequently, root vegetables contain the highest dry matter compared to green leafy vegetables. Forms of oxalates in the vegetable samples studied are shown in Table I. The total oxalate (watersoluble and insoluble fraction) contents of spinach, swiss-chard and beet root were the highest, 9794, 7554 and 222 mg/100g, respectively whereas contents of total oxalate in sweet-potato, potato, kale, lettuce and cabbage were low, the values being 59, 77, 79, 81 and 142 mg/100 g, respectively. The contents of water-soluble oxalates in lettuce and kale were equal (60 and 61 mg/100 g, respectively). However, the contents of water-soluble oxalates in spinach, swiss-chard and beet root were 7796, 4684 and 216 mg/100 g, respectively. Both potato and sweet-potato samples contain equal amounts of water-soluble oxalates (38 mg/100 g). Values for water-soluble oxalates were also similar for carrot and cabbage.

Higher percentage of water-soluble oxalates to total oxalate was observed in spinach, kale and lettuce which amounted to 80, 76, 76, respectively (Table I). The lowest percentage concentration of water-soluble oxalates to total oxalate was noted in sweet-potato (50%). For other vegetables this ratio, ranges from 52 to 64%.

The insoluble form of oxalate expressed as calcium oxalate was shown in Table 1. Spinach and swiss-chard contain as high as 4182 and 2907 mg/100 mg calcium oxalate, respectively. Sweetpotato and lettuce contained the least amount of insoluble oxalate (32 and 36 mg/ 100 g, respectively). Table 2 shows the content of calcium, iron and phosphorus in the vegetable samples. The iron content of the vegetable samples ranges from 0.5 to 7.2 mg/100 g, the highest value being obtained in spinach. With respect to phosphorus content higher values were recorded in lettuce, swiss-chard and spinach (76, 68 and 67 mg/100 g, respectively). Potato, carrot, and beet root contained the least amount of phosphorus. As indicated in Table 2 the calcium content of the vegetable samples varied from 26 to 489 mg/100 g. Spinach and swiss-chard were found to possess higher levels of calcium (489 and 417 mg/100 g). Lower concentrations of calcium were observed in potato, sweet-potato and carrot (26, 29 and 37 mg/100 g, respectively).

The ratio of calcium to total oxalate was less than 0.5 for the study samples except for kale, lettuce and sweet-potato. The lowest ratio of calcium to total oxalate was observed particularly for spinach and swiss-chard. The ratio of calcium to phosphorus was much higher in spinach (6.0), swisschard (4.5) and kale (3.9). Lower values were recorded for the rest of the vegetables (Table 2).

Discussion

In the vegetable samples studied, water-soluble and insoluble forms of oxalates were estimated although the ratio of water-soluble to total oxalate varied. Hodgkinson (10) similarly reported that both soluble and insoluble oxalates (calcium oxalate) may be present, but the ratio may vary considerably in different foods. The total oxalate content of : sweet-potato, potato, carrot, lettuce,

cabbage and kale was in agreement with values for fruits, tubers and vegetables reported elsewhere (17, 18) .Considerable variations in the oxalate content of plants can occur depending on the season, species, variety and soil nutrients (15,19,20,21). The influence lthese factors however, was not considered In, the present study. The oxalate content in spinach and swiss- chard was much higher compared with other vegetables studied. Poneros-Schneier and Erdman (22) indicated that spinach was rich in

Table 1: Mean values for dry matter, total oxalate, water-soluble oxalate in vegetables (mg/100 g dry weight)*

Vegetables	Dry matter, fresh weight	% of total oxalate	Soluble oxalate	Insoluble oxalate	Ratio of calcium oxalate	soluble oxalate/ Total oxalate, %
Beet root	11.2 \pm 2.1	221.6 \pm 6.3	216.2 \pm 3.4	216.2 \pm 3.4	0.25 \pm 0.01	55
Cabbage	8.7 \pm 1.3	141.6 \pm 7.2	73.1 \pm 7.2	98.9 \pm 2.9	0.36 \pm 0.02	52
Carro	9.8 \pm 1.0	114.5 \pm 4.4	71.4 \pm 4.4	63.4 \pm 1.5	0.32 \pm 0.01	62
Kale	12.9 \pm 3.5	79.3 \pm 4.7	60.1 \pm 3.5	27.8 \pm 1.5	2.80 \pm 0.1	76
Lettuce	6.4 \pm 4.6	80.5 \pm 6.3	61.3 \pm 3.2	35.5 \pm 1.8	1.20 \pm 0.1	76
Potato	24.3 \pm 3.1	77.0 \pm 4.0	38.2 \pm 1.6	46.1 \pm 1.7	0.34 \pm 0.05	50
Sweet Potato	28.5 \pm 1.7	59.0 \pm 6.3	38.0 \pm 1.3	31.7 \pm 3.7	0.49 \pm 0.02	64
Spinach	11.4 \pm 4.3	9793.7 \pm 16.3	7796.1 \pm 18.1	2906.5 \pm 11.2	0.05 \pm 0.01	80
Swiss Chard	8.3 \pm 1.7	7554.0 \pm 20.1	4684.1 \pm 15.9	4181.6 \pm 12.8	0.06 \pm 0.01	62

Table 2: Mean values for minerals in vegetables (mg/100 g dry weight)*

Vegetables	Total calcium	Free calcium	Phosphorus	Calcium/Phosphorus	Iron
Beet root	83.0 \pm 2.2	15.3 \pm 0.9	34.9 \pm 9.3	2.4 \pm 0.2	1.4 \pm 0.9
Cabbage	50.8 \pm 3.9	19.9 \pm 1.2	41.7 \pm 14.3	1.3 \pm 0.5	1.0 \pm 0.4

Carrot	36.7±2.4	7.8±0.9	34.8±8.8	1.0±0.3	1.3±0.4
Kale	221.8±4.8	213.1±2.8	52.2±9.1	3.9±1.3	3.4±0.5
Lettuce	98.5±1.9	84.5±3.1	75.9±15.3	1.3±0.3	3.6±0.2
Potato	26.1±1.0	11.7±1.1	25.5±7.7	1.1±0.1	5.7±0.8
Sweet potato	29.3±1.2	19.3±0.7	51.8±3.4	0.6±0.2	0.5±0.2
Spinach	488.7±3.6	Nil	66.5±15.5	6.0±1.3	7.2±2.8
Swiss Chard	416.9±6.6	Nil	68.4±13.8	4.5±1.8	5.4±0.7

a-Mean values of three analyses ± S.D., n = 14

oxalate, which was thought to inhibit calcium absorption by forming insoluble calcium oxalate salts in the small intestine. Vityakon and Standal (20) also reported high levels of oxalate in spinach.

During the analysis of oxalates, the extraction with boiling water dissolved water- soluble oxalates (sodium-, potassium- and ammonium-oxalate) .Spinach and ,swiss-chard followed by beet root contained higher levels of water-soluble oxalates. The water-soluble oxalates are comparatively more toxic as they form insoluble salts with calcium and magnesium rendering these trace metals unavailable from other sources of food in the digestive tract (2). Kansal and pahwa (23) also reported that spinach contained a very high amount of oxalates (11 g/100 g), 74.3% of which were in water-soluble form. Although the distribution of oxalate in our study samples varies, the study demonstrated , that green leafy vegetables contain higher oxalate content than other types of vegetables.

Vegetables are also good source of iron. Spinach contained high amount of iron compared to other leafy vegetables studied. Iron in vegetables however, forms stable but soluble complexes with oxalate which might be available for absorption. Van Campen and Welch (24) indicated that the high oxalate of spinach did not result in poor availability of iron at least to rats which means that spinach could be a good iron source.

The vegetables studied appear to be rich sources of calcium but not so nutritionally owing to high content of oxalates. For instance, while spinach contains high level of calcium, it is shown to be unavailable for absorption due to the formation of the insoluble calcium oxalate and this has been demonstrated in human studies (25). Kelsay (26) also reported that spinach is rich in oxalic acid, which inhibit calcium absorption by

forming insoluble calcium oxalate salt in the small intestine. Weaver (27) also suggested that the presence of spinach in a meal could reduce the availability of calcium from other food stuffs consumed during the same meal. Although spinach and swiss-chard contain higher level of calcium, the amount of free calcium available for utilization in the body was virtually zero. The ratio of calcium to oxalate less than 2.0 with the exception of kale indicates that all the calcium contained in these vegetables is bound to oxalic acid thus making it unavailable for absorption. Ackermann and Gepauer (28) suggested that the calcium/oxalate ratio in a diet just below the critical value of 2.0 indicates a likely excess in the intake of oxalates. In the present study, the calcium/oxalate ratio lies on the safe side of the toxic level in kale. This ratio was unfavourable in the other vegetables studied.

Kelsay (29) demonstrated that the calcium from oxalate containing vegetables has been shown to have lower biological value than calcium from vegetables not containing oxalic acid. The phosphorus content of the vegetables studied was found to be within the recommended limits for optimum utilization (30).

The relatively low concentration of total calcium compared with insoluble calcium oxalate suggest that most of the vegetables , tissue calcium was unavailable for absorption by the body. This insoluble oxalate cannot inactivate further calcium whereas the soluble forms can combine with calcium from other foods and reduce its availability .

A substantial variation was observed in the total oxalate content and its fractions in the vegetable samples. However, it may be possible to improve the nutritional value of these vegetables by reducing the soluble oxalate as it accounts for over 50% of the total oxalate present.

Acknowledgement

This study was financially supported by the Ethiopian Nutrition Institute.

References

1. Bekele A, Wolde-Gebriel Z, Kloos H. Food, diet and nutrition. In: Zein Ahmed Zein and Kloos, H(eds), The ecology of health and disease in Ethiopia. Westview Press, Oxford, 1988:85-102.
2. Pingle U, Ramassastri BV. Absorption of calcium from a leafy vegetable rich in oxalate. Br. I. Nutr. 1978;39:119-125.
3. Oke OL. Toxic chemicals in Nigerian foodstuffs. Ind. I. Nutr. Oietet. 1970;7:119-129.
4. Pirie NU. The production and use of leaf protein. Proc. Nutr. Soc. 1969;28:85-90.
5. SinghU, SaxenaNC. Effect of maturity on the oxalate and cation contents of six leafy vegetables. Ind. I. Nutr. Oietet. 1972;9:269-276.
6. Simmersbach F. Presenting micronutrient deficiencies: food abundance and diversity are fundamental. Food Nutr. Agric. 1993;7:8-17.
7. Gupta K, Wagle OS. Nutritional and antinutritional factors of green leafy vegetables. I. Agric. Food Chem. 1988;36:472-474.
8. Singh PP, Sharma NC, Sur BK. Value of green leaves as sources of available calcium. Ind. I. Med. Res. 1969;57:204-209.
9. Singh PP, Sharma OC and Saxena SN Nutritional value of foods in relation to the oxalic acid content. Am. I. Clin. Nutr. 1862;25:1147-1152.

10. Hodgkinson A. Oxalic acid in biology and medicine. London, Academic Press, 1977.
11. Asfaw Z, Nigatu A and Asefa M. Survey of the indigenous food plants of Ethiopia and food preparations from the indigenous food crops. Addis Ababa University , Addis Ababa, 1992.
12. Wolde-Gebriel Z, Oemeke T, West CE. Xerophthalmia in Ethiopia: a nationwide ophthalmological, biochemical and anthropometric survey. *Eur. J. Clin. Nutr.* 1991;45:469-478.
13. Selinus R, Gobezie A, Vahlquist D. Dietary studies in Ethiopia. 111. *Acta Sac. Med. Upsaliensis.* 1971;t:XXVI:158-178.
14. Knutson KE, Selinus R. Fasting in Ethiopia. *Am. J. Clin. Nutr.* 1970;23:956-959.
15. Kasidas GP, Rose GA. Oxalate content of some common foods; determination by an enzymatic method. *J. Hum. Nutr.* 1980;34:255-266.
16. AOAC. Association of Official Analytical Chemists, Washington DC.
17. Fiske CW, Subbarow Y. The colorimetric determination of phosphorus. *J. Biol. Chem.* 1925;66:375-400.
18. Dradbury JH, Holloway WD. Chemistry of Tropical Root Crops: Significance for nutrition and agriculture. Australian Centre for International Agricultural Research, Canberra, 1988;46-47.
19. Fasset DW. Oxalates. In: Toxicants Occurring Naturally in Foods. National Academy of Sciences, Washington DC. 1973;342-362.
20. Vityakon P, Standal DR. Oxalate in vegetable Amaranth. Forms, contents and their possible implication for human health. *J. Sci. Food Agric.* 1989;48:469-474.
21. Singh PP, Saxena NC. Variation in oxalate
22. Poneros-Schneier AG and Erdman JW. IR Bioavailability of calcium from sesame seeds, almond powder, white bread, spinach and nonfat dry milk in rats. *J. Food Sci.* 1989;54: 150-153.
23. Kansal VK and Pahwa A. Utilization of magnesium from leafy vegetables and cereals :effect of incorporating skim milk powder in the diets. *Ind. J. Nutr. Dietet.* 1979;16:453-459. ,
24. Van Campen DR and Welch RM. Availability to rats of iron from spinach:effect of oxalic acid. *J. Nutr.* 1980;110:1618-121.
25. Heaney RP, Weaver CM and Decker D. Calcium absorbability from spinach. *Am. J. Clin. Nutr.* 1988;47:707-711.
26. Kelsay IL, and Pratter ES. Mineral balance of human subjects consuming spinach in a low fibre diet and in a diet containing fruits and vegetables. *Am. J. Clin. Nutr.* 1983;38:12-19.
27. Weaver CM, Martin BR, Ebner IS and Krueger CA. Oxalic acid decreases calcium absorption in rats. *J. Nutr.* 1987;117:1903- 1905.
28. Ackermann H and Gebauer H. The toxicity of oxalate containing vegetables and feed plants. *Nahrung.* 1957;1:278-283.
29. Kelsay IL. Effect of oxalic acid on calcium bioavailability. In: Kies, C(ed.), Nutritional Bioavailability of Calcium. Washington DC, American Chemical Society , 1985: 105-113.
30. National Research Council (USA). Recommended Dietary Allowances. A report of the Food and Nutrition Board. Publ. No. 1964, Washington DC, National Academy of Sciences, 1968.