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Determination of oxalates and investigation of effect of boiling on oxalate content from selected vegetables commonly grow in Eritrea.

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ABSTRACT

Oxalic acid is one of the abundant organic compound acts as an anti-nutrient present in most vegetables in various quantities. It is found as salts of insoluble complexes with divalent cations, minerals and trace elements. Oxalates react with calcium to precipitate calcium oxalate and accumulation of oxalates in the body prevents the absorption and utilization of calcium; which in turn causes calcium imbalance, rickets and osteomalacia. In the present study oxalate content of various vegetables commonly available in local market of Asmara and Massawa were determined and effect of boiling on oxalate content of selected vegetable were investigated. Most of the samples analyzed were contained more than 100mg/100g on dry weigh basis. The higher value of total oxalate content was found in Okra (630.4mg), Tomato (472.80mg) and Spinach (471.60mg); Potato, Mustard, Rocket salad, Curette and Swiss chard contain above 250mg/100g; Cabbage and Lettuce contain below 200mg/100g. Boiling has shown reduction in total oxalate content and least reduction was obtained in Mustard leaves (5.88%) and highest reduction was obtained from Spinach (40.00%).

KEY WORDS: Eritrean vegetables, Oxalate, boiling, kidney stones.

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INTRODUCTION

Food is any substance consumed to provide nutritional support for human body. There are around 2,000 plant species are cultivated for food, and many have several distinct cultivar (McGee and Harolds, 2004). Vegetable is an important contributor to human diet and are good source of carbohydrate including starch and cellulose, large amount of vitamins folic

acid, vitamin A and vitamin C (Raheena, 2007). The plants are good sources of mineral elements like Iron, Calcium, Magnesium, Sodium, Phosphorus, Cobalt, Copper, Potassium, and Chlorine due to uptake from soil at various stage of growth. Vegetables are also poor sources of calories except in roots and tubers (Bakhru, 2007).

The main problem in nutritional exploitation of green leafy vegetable is the presence of anti-nutrients. Oxalate is one of the organic compounds found in plants and is an anti-nutrient. Oxalate is associated with metabolic disorders and infectious diseases (Holmes and Assimos, 1998; Nakagawa *et al*., 1999). The oxalate content of plants can vary according to their age, the season, the climate and the type of soil. In some plants, such as Rhubarb, oxalate content tends to increase as the plants mature, whereas, in other plants, e. g. Spinach, sugar beet leaves, and bananas, there is a large increase in oxalate content during the early stages of development, followed by a decrease as the plants mature (Horner and Wagner, 1995). In plants oxalates play role in ion balance, plant defense, tissue support, detoxification, light gathering and reflection (Franceschi and Horner, 1980).

The population of Eritrea has limited food habits, they consume some of the vegetable as raw salads and some are in boiled form. Usually vegetable accumulate high amount oxalates. Consumption these vegetables in raw or boiled form of can deposit large amounts of plant oxalates and its absorption can be fatal to both humans and animals; it causes oxalosis (Holmes *et al.,* 1995). Human urine always contains small levels of calcium oxalate; excess oxalates in body can trigger to increase urinary oxaltes that may be deposited in the kidneys as common form of kidney stones (Massey *et al.,* 1993; Oke, 1969). Sometimes it can cause acute renal failure in (Simpson *et al*., 1999). About 75% of all kidney stones are composed primarily of calcium oxalate (Williams and Wandzilak, 1989) and hyperoxaluria is a primary risk factor for this disorder (Goldfarb, 1988; Robertson and Hughes, 1993). Restriction of dietary oxalate intake has been proposed to prevent the formation of calcium oxalate kidney stones.

MATERIAL AND METHODS:

Sample Collection

Ten vegetable samples were selected from the local market of Asmara (Shuk) and Massawa (Edaga) for determination of oxalate content. The selected samples were Spinach, Lettuce, Mustard, Cabbage, Swiss chard, Potato, Okra, Tomato, Curette, and Salad rocket.

Preparation and analysis of sample:

All the selected samples were packed in a clean polyethylene bags and transferred to laboratory for processing. The samples were divided into two parts, 100g of clean sample was dried directly in an oven at a temperature of (45^0C) and another 100g of sample was boiled for 15 minutes in fresh water then the samples were kept in oven for drying at temperature of (45^0C) for 24 hours. After drying all the samples were pulverized to a uniform particle size and packed in an airtight container for further analysis.

Titration using KMnO4

The oxalate content in all the samples were analyzed by following titration method using $KMnO_4$ described in AOAC, 1990. In the determination of oxalate, 1g of each selected vegetable samples were weighed and mixed with 20 ml 0.1M HCl in a 50ml beaker to extract total oxalate and another 1g of each selected samples were weighed and mixed with 20ml of distilled water to extract soluble oxalate. All beakers with samples and

extracting solvents were kept in a water bath at 100^{o}C for 30 minutes, later filtrated using Wattman No 1 filter paper. 0.5ml of 5% Calcium chloride was added to the filtrate to precipitate out Calcium oxalate, the precipitate was separated by centrifugation at 3500 rpm for 15 minutes, and supernatant was discarded. The Calcium oxalate precipitate was washed with 2ml of 0.35M Ammonium hydroxide and then dissolved in 0.5M of Sulphuric acid. The dissolved solution was titrated with 0.1M of Potassium Permanganate at 60^{0}C till faint pink color was persisted for at least 15 seconds. The oxalate content was calculated by using stoichiometric formula. The soluble oxalate was subtracted from total oxalate to obtain insoluble oxalate; both soluble and insoluble oxalates were expressed on dry weigh basis (DW).

RESULT AND DISCUSSION:

The total oxalate, soluble oxalate and insoluble oxalate content of the selected ten samples were expressed in mg/100g of dry weight. The oxalate content of unboiled and boiled samples with percentage of reduction is given in table 2.

Table 1: Names of samples investigated in the present study

Scientific Name	English Name	Local Name	Part of Plant
Brassica juncea	Mustard	Hamli Adri (ሓምሊ ኣድሪ)	Leaf &Stem
Spinacia oleracea	Spinach	Spinach (ስፒናች)	Leaf &Stem
Brassica oleracea	Cabbage	Kawlo (ካውሎ)	Leaf & Steam
Lactuca sativa	Lettuce	Salata (ሰላጣ)	Leaf & Steam
Abelmoschus esculentus	Okra	Bamya (ባምያ)	Fruit
Solanum tuberasum	Potato	Dnish (ድኒሽ)	Root
Solanum lycopersicum	Tomato	Komidere (ኮሚደረ)	Fruit
Eruca sativa	Rocket salad	Jirjir (ጂርጂር)	Leaf & Steam
Cucurbita pepo	Curette	Zukini (ዙኪኒ)	Fruit
Beta vulgaris L. cicla	Swiss chard	Kosta (ቆስታ)	Leaf &Stem

Table 2 Oxalate content in selected raw vegetable samples on dry weight basis.

Scientific Name	Unboiled Samples(mg/100g)			Boiled Samples(mg/100g)			% Reduction of total oxalate
	Insoluble Oxalate	Soluble Oxalate	Total Oxalate	Insoluble Oxalate	Soluble Oxalate	Total Oxalate	
B. juncea	91.41	176.51	267.92	94.56	157.60	252.16	5.88%
S. oleracea	157.60	315.20	472.80	31.52	252.16	283.68	40.00%
B. oleraceCa	94.56	94.56	189.12	78.80	63.04	141.84	25.00%
L. sativa	31.52	157.60	189.12	31.52	141.84	173.36	8.33%
A. esculentus	126.08	504.32	630.40	94.56	441.28	535.84	15.00%
S. lycopersicum	129.23	154.45	283.68	94.56	126.08	220.64	22.22%
S. tuberasum	251.16	220.44	471.60	220.64	157.60	378.24	19.79%
E. sativa	47.28	283.68	330.96	94.56	157.60	252.16	23.80%
C. pepo	157.60	157.60	315.20	110.32	110.32	220.64	30.00%
B. vulgaris L. cicla	126.08	252.16	378.24	78.80	173.36	252.16	33.33%

Total oxalate content of the selected vegetable on dry weight basis of unboiled or raw samples was ranged from 189.12mg to 630.4 mg/100g. The lowest amount of total oxalate content (189.12mg/100g) was found in *L. sativa* and *B. oleracea* and the highest was in *A. esculentus* (630.4mg/100g) followed by *S. oleracea* (472.8mg/100g) and *B. vulgaris L.cicla* (378.24mg/100g). Mohammed and Luka (2013) determined total oxalate from *B. oleracea* collected from Nigeria contains 225mg of total oxalates/100g of sample. But the same species investigated in the present analysis has reported lower amount of total oxalates (189mg/100g) of dry weigh. The composition of nutrients and anti-nutrient in plants may vary depending on the variety and growing conditions. It is noticeable that each of the leaves can accumulate high in one or two anti-nutrient and low in another (Ruales, 1993).

The lowest level of total oxalate in boiled samples was obtained from *B. oleracea* (141.84mg) followed by *L. sativa* and *S. tuberasum* (173.36mg) and (220.64mg) per 100g respectively. *A. esculentus* (534.84mg) followed by *S. lycopersicum (*378.24mg) and *S. oleracea (*283.68mg)/100g recorded the high values of total oxalate in boiled samples.

Boiling has shown effect on oxalate content of all the samples investigated in the present study. The percentage of reduction of total oxalates was ranged from 5.88% to 40.00%. *B. junicea* has shown least percentage of reduction while *S. oleracea* has reported with highest percentage of reduction. Sharma and Sehgal (1990) have noticed the variation in phytochemical contents of fresh fruits and vegetables include genetics, species of plant, and stage of growth, harvesting and post-harvest handling during storage.

In Eritrea Tomato and Potatoes are highly consumed vegetables; Salata and Rocket salad are eaten as raw vegetables as vegetable salads and the other analyzed vegetables are eaten as cooked form. Tsai *et al*, (2005) have reported the mean lethal dose of oxalates for an adult is 15 to 30g, and the lowest reported lethal dose is about 70mg/kg body weight. Recent reports showed that the diet with high oxalates can interfere with calcium absorption in kidney and increase the possibilities to form kidney stones (Elinge. *et al*, 2012). However, the critical factor in stone formation does not necessarily correspond with oxalate content. According to Massey et al, (1993) certain foods such as Swiss chard, parsley and collards have high oxalate content but the bioavailability is low. Therefore though *B. vulgaris L.cicla* is having high oxalates (378.24mg/100g), the possibilities to cause kidney stones are low. The same sample in the present investigation has shown maximum percentage of reduction (33.33%) on cooking.

CONCLUSION

All the samples investigated in the present study contains considerable amount of both soluble and insoluble oxalate. The boiling of samples for 15 minutes have shown great reduction in the concentration of soluble oxalate and therefore total oxalates in some samples like *Spinacia oleracea* and *Brassica juncea. Abelmoschus esculentus*, *Spinacia oleracea*, *Solanum lycopersicum*, *Brassica juncea* and *Beta vulgaris L. cica* showed highest amount of oxalate and even after boiling the samples. Usually low oxalate diet should contain 50mg/100g and is the recommended daily intake per day. Too much of oxalate in food combine with endogenously synthesized oxalates to form urinary oxalates results in the formation of kidney stones. High concentration of oxalates in the body also prevents the absorption of soluble calcium ions and the oxalate bonds with the calcium ions to form insoluble calcium

oxalate complex. Therefore, people who have tendency to form kidney stones should avoid oxalate-rich foods.

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