5 Total antioxidant content in foodstuffs from arsenic-affected area of Bangladesh

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Abstract

Foodstuffs were collected from Chadpur District, Bangladesh to examine whether chronic exposure to arsenic modifies their total antioxidant content. Total antioxidant content was estimated using the ferric reducing antioxidant power (FRAP) method. The mean antioxidant content at arsenic concentrations less than 500 μg/kg varied between 1.03 to 7.05 mmol/100 g fresh weight, with the highest concentration found in guava and the lowest in raw rice. Higher accumulations of arsenic from <500 μg/kg to >1000 μg/kg did not alter the total antioxidant content of rice, green papaya, lady's finger, arum leaf, green chili, egg plant, lemon and guava. Amaranth leaf showed only 26.5% inhibition of total antioxidant content. all-E Lutein was the major carotenoid in Amaranth leaf. The amount of all-E lutein and all-E zeaxanthin decreased (28.2% and 64.9% respectively) in the presence of high concentrations of

arsenic. Our results suggest that the total antioxidant content of foodstuffs from an arsenic-affected area is not modified by the presence of high concentrations of arsenic.

Introduction

Dietary plants contain hundreds of antioxidants including ascorbate, carotenoids, reduced glutathione (GSH), tocopherols, polyphenolic acids, sulfides, flavonoids, lignans, lipoic acid and coenzyme Q_{10}, (Chaudiere and Ferrari-iliou, 1999). Epidemiological study shows that high intake of fruits and vegetables decreases the incidence of some chronic diseases such as cancer, coronary heart disease, hypertension, cataract and diabetes mellitus type 2 (Block et al, 1992; Steinmetz and Potter, 1996; Ness and Powles, 1997; Krichevsky, 1999; Landrum and Bone, 2001). However, conclusive results are not obtained when supplemented with β-carotene, retinal, and tocopherols (Anonymous, 1994; Hennekens et al., 1996). Instead they may act as pro-oxidant (Burton and Ingold, 1984; Zhang and Omaye, 2001; Moini et al, 2002). These antioxidants contribute less than 25% of total antioxidants in most dietary plants. Therefore, total antioxidant activity is more important than that of an individual antioxidant.

In arsenic-affected areas, high concentrations of arsenic are consumed not only by humans but also by animals and plants (Roychowdhury et al, 2003; Huq and Naidu, 2003; Das et al, 2004). Oxidative stress also exists in plant (Requejo and Tena, 2005). One of the important examples of oxidative stress is the programmed plant senescence, which is associated with increased lipoxygenase activity and increased production of mitochondrial peroxides (Leshem, 1988). It is not known whether chronic arsenic exposure affects the total antioxidant content as well as its nutrient value of dietary plants. The purpose of the present study is to examine whether chronic arsenic exposure modifies the total antioxidant content of foodstuffs.

Materials and Methods

Chemicals: 2,4,6-tri-pyridyl-s-triazine (TPTZ), all-E lutein, all-E β-carotene and all-E lycopene were purchased from Sigma Chemicals Company (St. Louis, MO, USA). Zeaxanthin was donated by Square Pharmaceuticals (Bangladesh). HPLC grade methanol and acetonitrile were purchased from Merck (Germany). Ferric chloride, sodium borohydride and arsenic trioxide were purchased from Loba Chemie (India). All the standards of carotenoids were stored at -°70C in solvent free conditions aerated with argon.

Collection of foodstuffs: Foodstuffs were randomly collected from sixty houses (using random number table) of an arsenic-affected area (Shahrasti Upazilla of Chadpur District, 140 km from Dhaka) in June 2005. Shahrasti Upazilla is one of the badly affected areas due to high concentration of arsenic in shallow tubewell water. More than 95% of the tubewells contain high concentration of arsenic. From each house, a maximum of 10 items of foodstuffs were collected. Food items having higher number of samples were included in this study. Foodstuffs included raw rice (*Oryza sativa*), guava (*Psidium guajava*), leaf of *Amaranthus gangeticus* (local name: data shak), green papaya (*Carica papaya*), lady's finger (*Hibiscus esculentus*), lemon (*Citrus limon*), arum leaf (*Colocasia antiquorum*), green chili (*Capsicum annum*) and egg plant (*Solanum xanthocarpum*). Among them aramanth, green papaya, lady's finger, arum leaf and egg plant are consumed as vegetables. The availability of vegetables varies in different seasons of Bangladesh. Each sample was collected in propylene bags and identified with a code number. All the samples were transported to the laboratory on the same day of collection and kept at -20°C prior to analysis for total arsenic, total antioxidant content and major carotenoids concentration.

Estimation of total arsenic: The amount of total arsenic was estimated using continuous flow hydride generator atomic absorption spectrophotometer (model 210 VGP, Buck Scientific Co., CT, USA) as described previously (Misbahuddin, et al., 2006). In brief, 200 mg of foodstuff was pulverized/chopped up, mixed with 5 ml of 50% methanol, and kept overnight at room temperature. After filtration, the extract was treated with potassium iodide and was introduced into the hydride generator by continuous flow of 10% hydrochloric acid, 3% sulfuric acid and 1% sodium borohydride into a gas-liquid separator. The arsine vapor produced by arsenic and the hydrogen gas (produced by sodium borohydride and acid) was carried by flowing argon gas into a quartz T-tube. The current of the hollow cathode lamp for arsenic was 10 mA. The wavelength was 193.7 nm and the limit of detection was 0.8 μg/l.

Determination of total antioxidant activity: Total antioxidant activity of foodstuffs was measured using ferric reducing antioxidant power (FRAP) method (Benzie and Strain, 1996). In brief: 200 mg of foodstuff was pulverized/chopped up and mixed with 1 ml of distilled water. Then 9 ml of methanol was added and sonicated in a water bath for 15 minutes. After centrifugation 0.1 ml of the extract was mixed with 3 ml of FRAP reagent (TPTZ 10 mM, hydrochloric acid 40 mM, ferric chloride 20 mM, sodium acetate buffer 300 mM, pH 3.6). After standing at room temperature for 4 minutes, the absorbance was taken at 593 nm using a spectrophotometer (model UV-VIS 1201, Shimadzu, Japan) against a reagent blank.

Determination of major carotenoids: Amaranth leaves were thawed, weighed, homogenized and analyzed for all-E lutein, all-E zeaxanthin, all-E lycopene and β-carotene by reverse phase HPLC following extraction procedures (Thumham et al., 1998). Two hundred milligrams of Amaranth leaves were chopped into small pieces and homogenized in 5 ml of water using a hand homogenizer. Carotenoids were extracted in the presence of 5 ml hexane: acetone: methanol (2:1:1; v/v) for 30 min. The extraction procedure was repeated three times. Butylated hydroxytoluene (0.05%) was added in both the extraction solvent and the mobile phase. Samples were then centrifuged (5,000 x g) for 10 min and the upper organic phase was removed and evaporated to dryness under nitrogen. The dry residue was dissolved using 100 μl of the mobile phase for HPLC analysis. The HPLC system consists of a liquid chromatography solvent delivery pump (model 582, ESA, USA), a metal-free six-port injector (Rheodyne, Rohnert Park, CA, USA) with a 20 μl sample loop, C_{18} column (250 x 4.6 mm, 5 μm particle size, ProntoSIL, Bischoff Chromatography, Germany) and a visible detector (model 522, ESA, USA). All the tubing in contact with the mobile phase was made of inert PEEK material. The mobile phase was an isocratic mixture of methanol: acetonitrile: chloroform (47:47:6; v/v) with a flow rate of 0.5 ml/min. The injection volume was 20 μl. Carotenoids were detected at 450 nm and identified by retention time compared with standards. The analysis was carried out at room temperature. The software (EZChrom Elite, Scientific Software Inc., USA) was used for peak integration and data acquisition. All analyses were carried out in duplicate with the mean repeated results should not vary by more than 10%. The limit of detection for all-E lutein, all-E lycopene and all-E β-carotene was 0.10, 0.13 and 0.003 ng respectively. Both extractions and analyses were performed under dimmed light to prevent photoisomerization and degradation of carotenoids.

Analyses of data: Statistical analyses were carried out using a standard software package (SPSS). The significance of differences in total antioxidant content of foodstuffs between the low arsenic group and moderate or high arsenic group was determined using a multiple comparison test (Dunnett's test). The Student's t-test was also performed where necessary. Statistical significance was determined by $p < 0.05$.

Results

Table 1 shows the total antioxidant content of foodstuffs according to their arsenic concentrations (less than 500 μg/kg, 501~1000 μg/kg and more than 1000 μg/kg). The mean antioxidant content of foodstuffs at arsenic concentrations less than 500 μg/kg varied between 1.03 to 7.05 mmol/100 g fresh weight, with the highest concentration found in guava and the lowest in rice. High accumulation of arsenic did not modify the total antioxidant content of rice, green papaya, lady's finger, arum leaf, green chili, egg plant, lemon and guava. A 26.5% inhibition of total antioxidant content was observed in the Amaranth leaf.

The amount of carotenoids in Amaranth leaf from the sample area was estimated (Table 2). The major carotenoid was all-E lutein, with small amounts of all-E zeaxanthin, all-E lycopene and all-E β-carotene. There were some additional Carotenoids that were not identified due to non-availability of commercial standards. Exposure to high concentration of arsenic (>1000 μg/kg arsenic) decreased the concentration of all-E lutein (28.2%) and all-E zeaxanthin (64.9%). These changes were statistically significant.

Table 1: Total antioxidant content of sample foodstuffs from an arsenic-affected area in Bangladesh

Foodstuffs	Local name	Arsenic range	Number of samples	Total antioxidant content* (mmol/100 g fresh weight)	P value
Raw rice	Chaal	<500 μg/kg	10	1.03 ± 0.48	-
		501~1000 μg/kg	31	1.10 ± 0.48	0.742[a]
		>1000 μg/kg	18	1.24 ± 0.85	0.893[a]
Green papaya	Kacha pepe	<500 μg/kg	4	2.30 ± 0.77	-
		501~1000 μg/kg	7	2.65 ± 0.54	0.903[a]
		>1000 μg/kg	3	2.08 ± 0.81	0.489[a]
Lady's finger	Dharash	<500 μg/kg	5	2.97 ± 1.70	-
		501~1000 μg/kg	7	2.82 ± 1.80	0.589[a]
		>1000 μg/kg	6	2.68 ± 1.07	0.599[a]
Egg plant	Begoon	<500 μg/kg	5	1.89 ± 1.22	-
		501~1000 μg/kg	6	3.40 ± 1.74	0.987[a]
		>1000 μg/kg	5	2.87 ± 1.06	0.942[a]
Lemon	Lebu	<500 μg/kg	7	3.12 ± 2.56	
		501~1000 μg/kg	11	5.14 ± 2.13	0.993[a]
		>1000 μg/kg	2	0.84 ± 0.30	0.191[a]
Amaranth leaf	Data shak	<500 μg/kg	26	2.12 ± 0.38	-
		501~1000 μg/kg	11	1.56 ± 0.23	0.002[b]
		>1000 μg/kg	0	-	-
Arum leaf	Kochu shak	<500 μg/kg	1	3.83 ± 0.00	-
		501~1000 μg/kg	8	4.24 ± 1.80	-
		>1000 μg/kg	11	3.73 ± 1.69	0.542[c]
Green chili	Kacha morich	<500 μg/kg	6	5.13 ± 1.45	-
		501~1000 μg/kg	10	5.56 ± 1.58	0.746[b]
		>1000 μg/kg	1	5.58 ± 0.00	-
Guava	Peyara	<500 μg/kg	10	7.05 ± 1.17	-
		501~1000 μg/kg	13	7.38 ± 2.25	0.817[a]
		>1000 μg/kg	11	7.92 ± 1.32	0.950[a]

*Data are mean ± SD; [a]when compared with the group containing <500 μg/kg arsenic using one-way ANOVA; [b]when compared with the group containing <500 μg/kg arsenic using student's t-test; [c]when compared with the group containing 501~1000 μg/kg arsenic using student's t-test

Table 2. Amount of major carotenoids in the Amaranth leaf

Arsenic range	Number of samples	Amount of carotenoids (μg/g fresh weight)*			
		all-E lutein	all-E zeaxanthin	all-E lycopene	all-E β-carotene
<500 μg/kg	16	15.19 ± 1.79	1.28 ± 0.46	0.24 ± 0.09	0.02 ± 0.02
501~1000 μg/kg	12	14.49 ± 0.70	0.68 ± 0.19	0.22 ± 0.11	0.03 ± 0.04
>1001 μg/kg	7	10.91 ± 1.51	0.45 ± 0.23	0.22 ± 0.24	0.02 ± 0.02

*Data are mean ± SD

Discussion

Our data shows that raw rice had the lowest level of antioxidants and guava had the highest (7-fold higher). The antioxidant content of rice, a staple crop, is very important as because it comprises about 69% of most Bangladeshi's food intake by weight (Chen et al, 2004). It is accompanied with vegetables (15.8%), lentils (daal) and a little fish, beef, mutton or chicken (4%) cooked in a spicy sauce. Consumption of fruits (1.7%) is not regular. So, the intake of total antioxidant largely depends on the amount of rice and vegetables. The Norwegian diet contributes only 19.6% of total antioxidant intake from cereals and vegetables (Halvorsen et al, 2001). Among the foodstuffs examined by Halvorsen et al, the lowest amounts of antioxidants were present in rice. However, the amount of total antioxidants in Norwegian rice was much lower than the Bangladeshi rice. In contrast, Miller et al (2000) shows that the antioxidant content of rice, on average, was 2 to 3 times higher than fruits, 2.5 to 6 times higher than vegetables and about 75% that of different varieties of berries. Intensely colored vegetables like red cabbage, red onion have high antioxidant content whereas watery vegetables such as potato, cucumber have very low value (Stratil et al, 2006). Guava, common fruit in Bangladesh, contained very high concentration of antioxidants.

Patients suffering from chronic arsenic poisoning with skin manifestations respond to antioxidant supplement (Ahmad, 1998; Bangladesh Arsenic Control Society, 2003). At least six months antioxidant supplement is required for complete recovery. Foodstuffs contain hundreds of antioxidants. Our result shows that the antioxidant content of foodstuffs from an arsenic-affected area are not generally affected. Therefore, screening is required to examine the total antioxidant contents of all the foodstuffs from arsenic-affected area. The total antioxidant content of foodstuffs was estimated using the FRAP method. This is a good method for estimating the total antioxidant content of dietary plants (Halvorsen et al, 2001). The validity of using this method in estimating total antioxidants in plasma is not well established because the FRAP method cannot estimate the thiol compounds.

A wide variety of amaranth leaves are consumed in many developing countries as green leafy vegetables. The type and amount of carotenoids varies and depends on factors like species, growing season and location. Amaranth leaves contain lutein, β-carotene, zeaxanthin and α-carotene. The amount of lutein is higher than the β-carotene (Haskell et al., 2005; Kidmose et al. 2006). Similar results were obtained in our study. The amount of all-E zeaxanthin and all-E lycopene were several fold higher than β-carotene but the amounts were not recorded in other studies. No β-cryptoxanthin was detected. Chronic exposure to arsenic reduces the total antioxidant content of amaranth, which may be due to low amounts of all-E zeaxanthin and all-E lutein. all-E Zeaxanthin is more affected than all-E lutein. Reduced levels of all-E zeaxanthin may be due to either inhibition of zeaxanthin synthesis or increased utilization more than under normal conditions.

The present study suggests that the total antioxidant contents of most of the foodstuffs tested, except amaranth, are not modified after chronic exposure to high concentrations of arsenic. However, the number of samples of analyzed foods was small, and screening of all the major foodstuffs from arsenic-affected areas is necessary to determine which foods have high antioxidants and could be promoted for regular consumption instead of supplementing with beta-carotene, ascorbic acid or alpha-tocopherol.

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