AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 33, NUMBER 2 JUNE 2004

ISSN 1116—4077

Afr. J. Med. med. Sci. (2004) 33, 171-175

Report

Ascorbic acid and mineral availability in two Nigerian plant foods.

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Summary
This paper reports the effect of various concentrations of ascorbic acid on the availability of Fe, Zn, Ca and Mg in two popular plant foods – cowpea and amaranthus vegetable – in Nigeria. Ascorbic acid enhancement of iron availability was over 300 % and zinc by 200 % from 0 – 100 mg concentration. Availability of iron was further increased by 200 mg ascorbic acid in amaranthus but showed a 50 % decrease in the legume. Availability of zinc was decreased by 200 mg ascorbic acid but to different levels in both plant foods. In the legume, maximum enhancement of Ca and Mg availability was exhibited at 100 mg ascorbic acid level but suppressed at higher concentrations. In amaranthus, maximum Ca enhancement was exerted by 200 mg ascorbic acid and 50 mg for Mg. Enhancement of Cu in the legume was marginally affected by ascorbic acid concentrations while inhibition of Cu was observed in amaranthus between 50-300 mg ascorbic acid concentrations. The effect of ascorbic acid on the availability of minerals seems to be concentration dependent and varies with the plant food.

Keywords: *Ascorbic acid, legume, amaranthus vegetable, mineral availability.*

Résumé
Ce project rapporte different concentration d'acide ascorbique sur la presence de fer, calcium et magnesium dans 2 types populaire d'haricot et de legumes au Nigéria. L'augemention de l'acide ascorbique sur la presence du fer était plus de 300% de concentration de 0-100mg. La presence du fer était plus elevée de 200 mg d'acide ascorbique en Amarranthus. Mais montrait 50% de reduction en legumes. La presence du zinc rédusait de 200 mg d'acide ascorbique mais un taux different entre les 2 plantes. Dans le légume, l'augmentation maximale du calcium et était monté de 100 mg d'acide ascorbique mais une réduction en concentration. Dans l'amaranthus, le taux d'augmentation du Ca était de 200 mg d'acide ascorbiqe et 50 mg de mg. L'agmentation de Cu dans le légume était marginalement affectée par la concentration d'acide ascorbique. Cependant l'inactivité du cuivre était observé dans la concentration d 'amaranthus entre 50-300 mg d'acide ascorbique. L'effect de l' acide ascorbique sur la presence des minéraux semble être dépendent de la concentration et varie en function de la plante.

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Introduction

Ascorbic acid has been known to enhance iron absorption from different types of food in human subjects [1,2] to reduce the concentration of soluble copper in the small intestinal lumen of rats [3] and does not seem to influence the bioavailability of inorganic zinc in man [4]. The mechanism of action of ascorbic acid has been postulated as the reduction of ferric to ferrous iron (enhancement) while copper availability may be decreased because of its reduction to cuprous species in the oxygen-free environment of the intestinal lumen [5]. On the other hand, [2] suggested that ascorbic acid promotes iron absorption from the diets by preventing the binding of iron by certain ligands such as phytate and tannin present in the diets.

Hazell and Johnson [6] observed a steady increase in the level of diffusible iron when different levels of ascorbic acid up to 300 mg was added to white wheat flour composite diets. Hallberg *et al.* [2] observed that ascorbic acid up to 50 mg enhanced iron availability in human subjects in a dose dependent fashion but the level of enhancement varied with the type of meal tested. The Nigerian diets are mainly of plant origin and the level of ascorbic acid needed for optimal increase in iron availability is not yet determined. Though ascorbic acid has been claimed to enhance calcium absorption [7], in the course of our studies, we observed some inhibition in the availability of calcium and zinc when 300 mg ascorbic acid was added to the cowpea meal [8].

In this era of megavitamin supplements and in Nigeria where orange flavored ascorbic acid tablet is taken as a confectionery ("sweet"), there is the need to study the effect of various concentrations of ascorbic acid on mineral availability from major sources of protein in order to determine optimal level of usage.

Materials and methods

Materials: The cowpea (IT86^{D}-1010) was obtained from the International Institute of Tropical Agriculture, Ibadan and kept in the freezer until used. The cowpea was cooked in twice its volume of water and mashed with the broth when it was soft to touch. The sample was then dried in the oven at 50°C and kept in the freezer until needed. Amaranthus vegetable was bought in a local market in Ile-Ife. The leafy part of the vegetable was washed in distilled water and blanched in boiling distilled water for five minutes, diced, and kept in a polythene bag in the freezer if not used immediately.

Reagents: All the reagents, obtained from Sigma except where specified and all other reagents used were of analytical grade. The glassware was washed, rinsed in dis-

tilled water, soaked overnight in 1.0 M HCl and rinsed thoroughly again with distilled water [9]. Pepsin and pancreatin solutions and the protein precipitant, a mixture of trichloroacetic acid and hydroxylamine hydrochloride (M & B, Dagenham), were prepared [9].

Analytical procedure: Moisture content: Two grams of each sample used in this study was dried in the oven at 105°C to constant weight according to the Association of Official Analytical Chemist (AOAC, 1984) method. Total mineral content was determined by the method of Akinyele and Osibanjo [10]. Determination of available minerals was carried out by a combination of the methods of Hazell and Johnson [6] and Miller *et al.* [9] with some modifications as follows:

(a) *Preparation of test meals*: The wet weight equivalent of six gram dry weight of the samples was mixed with distilled water (depending on the moisture content of the sample) to give a 150 g meal. The mixture was homogenized in a Kenwood KW 10 food blender to a creamy consistency, adjusted to a pH of 2 with 6 M HCl, divided into 50 g portions and frozen until processed following steps b, c and e below.
Preparation of Composite meals: Various weights - 50, 100, 200, 300, and 400 mg - of ascorbic acid were mixed with the wet weight equivalent of 6 g dry weight of blanched amaranthus vegetable and cowpea IT86D-1010 and the process indicated in (a) above was followed.

(b) *Simulated digestion of test meals:* Pepsin-HCl digestion: The frozen meal from (a) above and the composite meals were thawed at 37°C and divided further into 25 g aliquots. Pepsin was added to provide 0.125 g per 25 g meal and then incubated in a Buchi water bath (model no 887196; type B 465) for 2 h at 37°C. After the incubation period, one of the 25 g portions was analyzed for titratable acidity while the other portion was frozen until used.
Pancreatin digestion: Pancreatin digestion: The frozen 25 g pepsin digest from (b) above was thawed and placed into a 100 ml beaker. Dialysis tubing (12,400 molecular weight cut off obtained from Sigma) which contained 10 mL distilled water and an amount of $NaHCO_3$ equivalent to the measured titratable acidity was placed into the beaker containing the 25 g pepsin digest sample. The beaker was sealed with parafilm and incubated in a Buchi water bath model No 887196, type B 465 at 37°C with continuous agitation until the pH was about 5 (approx. 30 min).
Pancreatin-bile extract mixture: Pancreatin-bile extract mixture (6.25 mL) was added to the beaker and incubated for 2 h with gentle shaking [9]. At the end of the incubation period, the dialysis tubing was washed with distilled water and volume of the dialysate noted and then frozen until used.

(c) *Determination of titratable acidity:* This was accomplished by the method of Miller *et al.*[9].

(d) *Determination of total mineral content:* Total mineral content was determined from 4 g wet weight of the vegetable and 0.5 g of the cowpea weighed in triplicate. 10 mL conc. HNO_3 was added to each sample in a digestion flask and allowed to stand overnight. The samples were heated carefully until the production of brown nitrogen (IV) oxide fume has ceased. The flasks were cooled and (2 - 4 mL) of 70 % perchloric acid was added. Heating was continued until the solutions turned colorless. The solutions were transferred into 50 mL standard flasks and diluted to mark with distilled water. Total mineral content was then analyzed by ALPHA 4 Atomic absorption spectrophotometer.

(e) *Estimation of available Fe, Zn, Cu, Ca and Mg*: Protein precipitant (2 ml) was added to 2 ml of the dialysate from the pepsin-pancreatin-bile extract digest, heated in boiling water for 10 min, cooled and centrifuged [11]. The supernatant was diluted as required and ionizable minerals determined as in (d) above.

(f). *Statistical analyses*: The results were expressed as mean of three determinations and standard deviations.

Results

The total mineral content of both Amaranthus vegetable and the legume IT86^D-1010 is presented in Table 1 while the effect of various concentrations of ascorbic acid on *in vitro* mineral availability from the legume (IT86^{D}1010 cowpea) and the most commonly consumed vegetable in Nigeria is presented in Figures 1–5. All the concentrations of ascorbic acid in this study significantly ($P<0.05$) increased iron availability from the composite diets when compared to the test meal alone (Figure 1). It is interesting to note that 100 mg ascorbic acid produced the most significant increase in iron availability in the legume diet (234 % increase) while 200 mg produced the highest increase in availability (390 %) in the amaranth vegetable. There was however a similar increase in the availability of iron with 100 mg ascorbic acid in both samples (290 % and 230 % increase in ama-

Table 1: Crude protein (CP %) and mineral content of cowpea IT86D-1010 and blanched amaranthys vegetable (mg/Kg dry weight)

Sample	CP(%)	Fe	Za	Cu	Mg	Ca
Cowpea	29.3 ± 0.2	56 ± 2.9	62 ± 3.7	12.2 ± 0.3	1075 ± 19.3	890 ± 10
Amaranthus	18.7 ± 0.7	464 ± 66	318 ± 2.5	24 ± 2.6	9100 ± 760 6	500 650

ranth and legume diets with 100 mg ascorbic acid respectively). The availability of zinc increased significantly ($P<0.05$) with ascorbic acid concentration up to 100 mg above which there was a sharp drop in availability in both cases (Figure 2). The difference in the effect of ascorbic acid on the two meals became apparent with copper. Figure 3 indicates that 50 mg of ascorbic acid significantly increased ($P<0.05$) available copper in the legume while drastically inhibiting same in the amaranth vegetable. At concentrations above this threshold, copper availability reduced in the legume meal while increasing in the amaranth meal. The availability of calcium increased in the legume with 100 mg ascorbic acid but further increase in ascorbic acid concentration thereafter inhibited the availability of calcium by over 65 %. In amaranth vegetable, there seems to be an appreciable inhibition of calcium availability at 50 mg concentration, an enhancement up to 200 mg ascorbic acid and a gradual decrease (Figure 4). 50 mg ascorbic acid enhanced magnesium availability by 47 % in the amaranth vegetable while the same increase was obtained from 100 mg ascorbic acid in the legume. Concentrations of ascorbic acid above these levels seemed to reduce the availability of magnesium (Figure 5).

Figure 1: Effect of Ascorbic Acid on Iron Availability

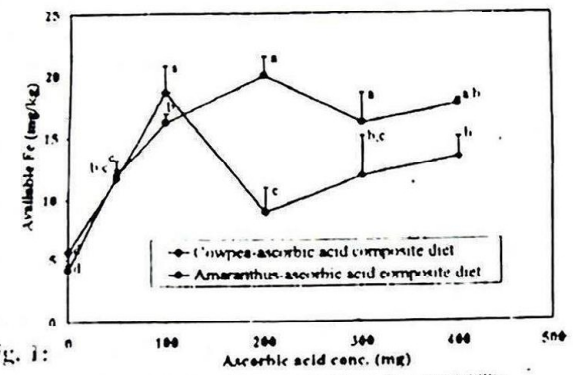

Fig. 1:

Figure 2: Effect of Ascorbic Acid on Zinc Availability

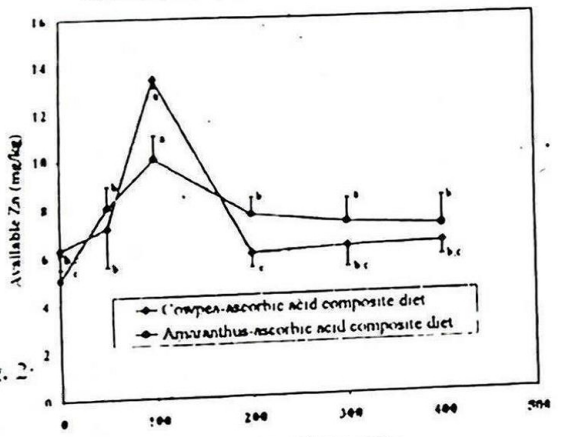

Fig. 2:

Figure 3: Effect of Ascorbic Acid on Copper Availability

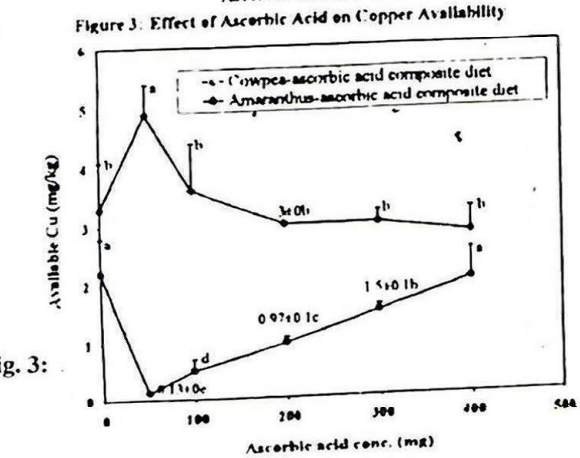

Fig. 3:

Figure 4: Effect of Ascorbic Acid on Calcium Availability

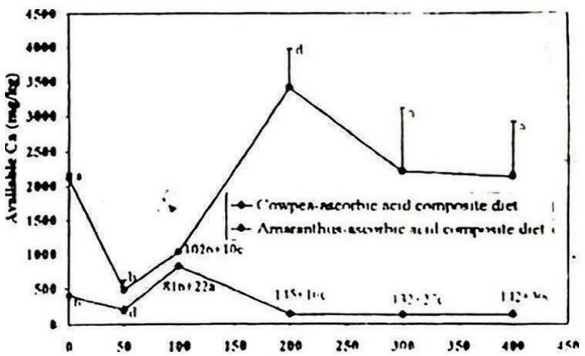

Fig. 4:

Figure 5: Ascorbic Acid Effect on Magnesium Availability

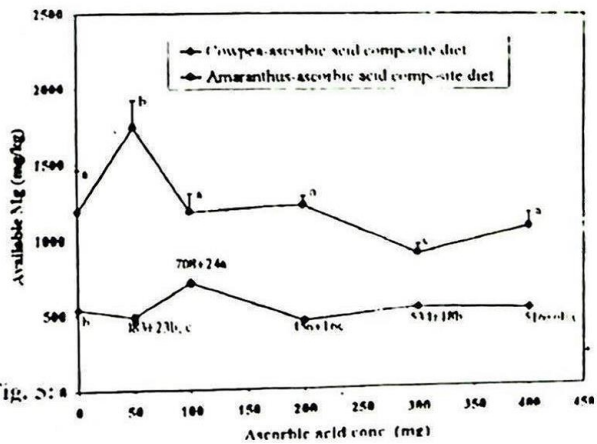

Fig. 5:

Discussion

The in vitro method of assessing mineral availability in plant foods is a reliable and sensitive method which has been found to correlate significantly with in vivo method using hemoglobin depleted rats [2,9,12,13]. Results presented herein could therefore be an accurate reflection of the availability of the minerals (at least iron) and highlight the effect of ascorbic acid on the mineral availability from the two plant foods.

It was observed in this study that the addition of ascorbic acid to a legume enhanced iron and zinc availability whereas that of calcium and magnesium was inhibited. A similar trend was also observed with decoction of *H. sabdariffa*, with high ascorbic acid content [8]. The effect of ascorbic acid on different foodstuffs has been shown to be dose dependent at least for iron availability [2] therefore effort was made to establish a relationship between various concentrations of ascorbic acid and mineral availability of two Nigerian foodstuffs.

The results now presented indicated that iron availability was not the same for different diets and was not strictly dose dependent, but varied with the composition of the diet. For instance, the highest level of iron availability was reached when 100 mg of ascorbic acid was added to the legume meal while a similar increase in availability was made possible by 200 mg ascorbic acid in the amaranth composite diet. Though higher levels of ascorbic acid enhanced the availability of iron from both foodstuffs when compared to the control (without ascorbic acid), the fact remains that there seems to be an optimum concentration or level of ascorbic acid for the attainment of maximum enhancement of iron availability. 100 mg of ascorbic acid seems to be the critical concentration at which all minerals were enhanced from the legume (IT86^{D}1010) so additional intake of ascorbic acid may be counter productive at least for the availability of iron.

Hallberg *et al.* [2] reported that 50–100 mg ascorbic acid added to the meal would cause maximum enhancement of iron availability because the effect of naturally occurring ligands such as hydroxyl ion, phosphate, phytate and tannin will be counteracted. On the other hand, Fe (III) may be reduced to Fe (II) by ascorbic acid [5] thereby enhancing Fe availability. Whatever the situation, it would be expected that an increase in the level of ascorbic acid would lead, in turn, to increased enhancement of iron availability which was contrary to what was observed in this study. It is therefore necessary to establish the concentration at which enhancement is maximum with different types of diets.

Availability of Zinc: It had earlier been observed that ascorbic acid had no influence on the bioavailability of inorganic zinc in man [4]. Our result however indicated that 100 mg ascorbic acid enhanced zinc availability from both legume and amaranth vegetable diets while higher levels of ascorbic acid also enhanced zinc availability though not to the same degree.

Availability of Copper: Ascorbic acid had been reported to reduce the concentration of soluble copper in the small intestinal lumen of rats [3] probably by reducing the Cu (II) to Cu (I) thereby precipitating the cuprous compounds. In the present study, 50 mg ascorbic acid enhanced copper availability in the legume but inhibited its availability in the amaranth vegetable. Beyond the 50 mg ascorbic acid level, the effect seemed to reverse in the two meals (Figure 3) indicating an unpredictable trend for copper availability.

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Received: 30 October 2002
Accepted: 8 April 2004