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The Level of Organic Acids in Some Nigerian Fruits and their Effect on Mineral Availability in Composite Diets

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Abstract: The pH, ascorbic, citric and total organic acid content of some local fruits in Nigeria were determined. The mineral content of the fruits, amaranthus vegetable and cowpea (*Vigna unguiculata* L. Walp) were also determined. Orange juice had the highest level of ascorbic acid but low in citric acid while lime juice is very rich in citric acid. Pine apple juice contained a low level of the organic acids. The effect of the juice from the different fruits on the amaranthus and cowpea composite diets were investigated and correlated with the acids content of the fruits. Orange and grapefruit enhanced Fe and Cu from both amaranthus and cowpea but seem impaired by pine apple and lime juice. All fruit juices enhanced Mg and Zn availability from amaranthus vegetable and cowpea composite diets except that Zn was inhibited by all the fruit juice from cowpea meals. There was generally a strong correlation between ascorbic, citric and total organic acid content of the fruits and the enhancement of mineral availability.

Key words: Ascorbic acid, citric acid, amaranthus, cowpea, mineral availability

Introduction

Minerals play a vital role in the maintenance of human health (Schrimshaw, 1991). Iron, for instance, is an important component of blood and enzymes involved in electron transfer. Its deficiency results in fatigue, headache and sore tongue in addition to anemia. Calcium is needed for bone formation while zinc is essential for protein and nucleic acid synthesis, carbohydrate metabolism, successful pregnancy, delivery and normal development (Wintrobe and Lee, 1974). Magnesium serves a key role in most reactions involving phosphate transfer, structural stability of nucleic acids and intestinal absorption of nutrients (Battiflora *et al.*, 1968).

In most African and other developing countries, mineral deficiency especially that of iron is still a public health issue probably due to the over dependence on plant food sources, which contain more than enough minerals to meet the daily requirement of man but have a low bioavailability for physiological purposes (Adewusi and Falade, 1996; Adewusi *et al.*, 1999). The low bioavailability of minerals from plant foods has been attributed to the presence of anti-nutritional factors such as tannin, phytate and oxalic acid (Awoyinka *et al.*, 1995; Santamaria *et al.*, 1999) while ligands such as ascorbic and other organic acids (Adewusi *et al.*, 1999; Hazell and Johnson, 1987) and some amino acids have been reported to enhance mineral bioavailability (Reinhold *et al.*, 1981).

Fruits, such as oranges, banana, grapefruit and pine apple abound in the tropical environment of Africa and other developing areas of the world and are consumed heavily when in season because storage technology is not available to preserve the excess production. Fruits contain organic acids especially ascorbic and citric acids with the latter predominating. Hallberg *et al.* (1986) reported the optimum concentration of ascorbic acid in iron enhancement to be 50 mg per meal while unpublished results from our laboratory indicated 100 mg ascorbic acid as the optimum concentration to enhance iron availability in legume and vegetable samples. Ascorbic acid has no effect on the absorption of inorganic zinc (Solomons *et al.*, 1979) while its effect on copper availability has been reported to be negative (Van den Berg *et al.*, 1994). In addition to its limited culinary use, lime is also used as a solvent medium in many local herbal preparations for cough, cold, indigestion and gastroenteritis especially for children. Only a limited number of studies have been initiated into tropical fruits, their chemical composition and the possible effect of the juices on mineral availability from composite diets of plant origin. This report therefore represents the first attempt in the series of investigations to bridge the gap in the knowledge of Nigerian fruits and their probable role in nutrition in the tropics.

Materials and Methods

Ripe fruits [Orange (*Citrus sinensis)*, Grape fruit (*Citrus paradisi*), Lime (*Citrus aurantifolia)*, Pine apple (*Ananas comosus*)] and *Amaranthus esculentus* vegetable were bought in the local markets in Ile-Ife. Cowpea (*Vigna unguiculata* L. Walp IT82D-699) was donated by the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The fruits were washed with distilled water, peeled, cut opened and the juice extracted. The sieved juice was stored frozen at -15 °C in a plastic container until used. The vegetable and cowpea were prepared as previously described (Adewusi *et al*., 1999; Adewusi and Falade, 1996).

Analytical Procedure:

a Moisture content was determined by the Association of Official Analytical Chemist (AOAC, 1984) method.

b Ascorbic acid was determined by the method of Roe and Kuether (1943) with some modifications thus: Five gram wet weight of each sample was extracted with 25 mL of 0.1% oxalic acid, the residue washed with 10 mL extraction solvent twice and the extract centrifuged and made up to 50 mL in a standard flask. The extract was thoroughly mixed with 4% trichloroacetic acid (ratio 1:9) and 0.75 g acid washed Norit and filtered through a No 1 Whatman filter paper. 0.5 mL of 10% thiourea solution was added to 1.0 mL of the filtrate, followed by 0.5 mL of 2.0% 2,4-dinitro phenylhydrazine (DNP) reagent in 4.5 M H_2SO_4. The test tubes were incubated in a Buchi water bath (Model No. 887196; Type B465) and incubated at 37 °C for 3 h and cooled in ice. To each test tube was added 2.5 mL of 85% H_2SO_4. The tubes were shaken thoroughly in ice and left for 30 min for colour formation. Absorbance was read at 540nm using a Pharmacia LKB spectrophotometer against a reagent blank prepared by adding the DNP reagent after the addition of 2.5 mL 85% H_2SO_4. A standard curve of ascorbic acid 0-100 mg per liter was established.

c Determination of Citric Acid Content was carried out by the UV method using the Boehringer Mannheim kit catalog No. 139076.

d **Determination of Total Titratable Acidity:** 20 mL aliquot of clear juice was titrated in a 250 mL Erlenmeyer flask against 0.3 M NaOH solution to an end point of pH 8.1 using a PYE Unicam pH meter (Model 290 MK 2). Total organic acid was calculated as the anhydrous citric acid equivalent.

e **Determination of Total and Available Mineral Content:** Total mineral content was determined from 4 g of each of the fruit juice and vegetable samples 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.

Available mineral content was determined by method of Miller *et al.* (1981) with some modifications.

(i) Preparation of test meals: 20 g fresh sample was mixed with 80 g water to make 100 g meal (fruit juice or amaranthus) while 10 g of cowpea (dry weight) was mixed with 90 g of water to make a 100 g meal. The mixture was homogenized in a Kenwood KW 10 food blender to a creamy consistency, adjusted to 40 g meal aliquots and frozen until used.

(ii) Simulated digestion of test meals: Pepsin-HCl digestion: The frozen meal from (i) above was thawed at 37 °C and divided into 20 g aliquots. Pepsin digestion was continued as previously described (Adewusi *et al*., 1999).

Pancreatin digestion: The frozen 20 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 20 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 pH was about 5 (approx. 30 min). Pancreatin-bile extract mixture (6.25 mL) was then added to the beaker and incubated for 2 h with gentle shaking. The volume of the dialysate was noted and then frozen until used.

(iii) Preparation of composite test meals: 16 g fruit (wet weight) was separately mixed with 4 g (dry weight) of vegetable or cowpea. Water was added to the mixture to make a 100 g meal and then blended to a creamy consistency. The resulting mixture was treated as in (a) and (b) above.

Determination of titratable acidity was carried out as described by Miller *et al.* (1981).

(iv) Estimation of available mineral: Protein from the dialysate was precipitated as previously described (Adewusi *et al.*, 1999) and the supernatant was diluted as required for available mineral determination using ALPHA 4 Atomic Absorption Spectrophotometer.

Statistical Analysis of Data: The results were expressed as a mean of three determinations with the exception of dietary fiber and available minerals that were presented as the mean of four determinations ± SD. Correlation analysis was carried out using the Pearson test.

Results and Discussion

The moisture content presented in Table 1 ranged between 87 and 92% for fruit juice samples, which agreed with arrange of 82 to 90% reported earlier (FAO,

Table 1: Percentage Moisture, pH value, Ascorbic, Citric and Total Organic Acid (mg/100 g) Content of Fruit Juice, Amaranthus Vegetable and Cowpea*

Sample	% Moisture	pH	Ascorbic acid[a]	Citric acid[b]	Total organic acid[c]
Pineapple	87.3 ± 1.5	3.5	11.7 ± 1.5	218 ± 4.0	246 ± 6.3
Orange[d]	90.2 ± 0.5	3.5	55.3 ± 1.5	452 ± 31	497 ± 5.2
Grape fruit	92.3 ± 0.7	3.2	45.4 ± 1.2	1312 ± 18	1382 ± 39.4
Lime[e]	91.2 ± 0.5	1.4	29.4 ± 1.4	4124 ± 78	4187 ± 35.1
Amaranthus[f]	89.7 ± 0.5	-	-	-	-
Cowpea[g]	10.6 ± 0.4	-	-	-	-

*Mean ± standard deviation of quadruplicate analysis expressed on wet weight basis.

a. expressed in mg/100 g
b. expressed in mg/100 g (expressed as anhydrous citric acid)
c. Total organic acid expressed as anhydrous citric acid/100 g juice.
d. The pH of unripe oranges plucked for transportation was 2.7 and the total organic acid content was 561mg/100 g juice.
e. pH, Ascorbic, Citric and total organic acid content could vary by up to 29% depending on the stage of fruit maturity and ripeness.
f. values are on wet weight basis (balanced vegetable)
g. Sample was cooked and dried at 50 °C prior to analysis.

Table 2: Total and Percent Available Iron Content of Some Fruit Juice, Amaranthus Vegetable, Cowpea together with the Theoretical and Experimental Available Iron of the Composite Diets[1]

Sample	Total Iron (mg/kg)	% available Iron	Available iron (mg/kg) in fruit-amaranthus and fruit-cowpea composite diets					
			Fruit Juice + Amaranthus[2]			Fruit Juice +Cowpea[3]		
			Theoretical	Exptal	%Df[4]	Theoretical	Exptal	%Df[4]
Pineapple	4.8 ± 0.9	48	3.9 ± 0.1	3.6 ± 0.8	-8	4.2 ± 0.1	3.2 ± 0.5	- 24
Orange	8.9 ± 1.2	32	2.6 ± 0.2	3.4 ±0.4	31	2.9 ± 0.2	3.4 ± 0.8	17
Grape fruit	10.7 ± 1.8	31	4.1 ± 0.1	4.5 ± 0.1	10	4.6 ± 0.2	6.9 ± 0.6	50
Lime	4.2 ± 0.7	31	7.0 ± 0.7	6.4 ± 1.0	- 9	5.6 ± 0.6	5.4 ± 0.5	-4
Amaranthus	86 ± 1.4	12	-	-	-	-	-	-
Cowpea[5]	157 ± 1.4	3	-	-	-	-	-	-

[1]Mean±standard deviation of quadruplicate analysis. [2] Composite diet = fruit juice and amaranthus mixed in ration 4:1. [3]Composite diet = fruit juice and cowpea mixed in ratio 9:1. [4]Df = difference between theoretical and experimental values. [5]Sample was cooked and dried at 50 °C prior to analysis.

1968; Beyond vegetarianism, 1994). The moisture content of amaranthus was equally high (85.4%). This value agreed favorably with 86.7% reported for amaranthus (Awoyinka *et al*., 1995). The pH of the fruit juices was between 1.4 and 3.8 (Table 1) which compared well with earlier values (Lark, 2001). The pH of the juice is dependent on the maturity and stage of ripeness of the fruits as indicated in Table 1 for oranges. Ascorbic acid content was least in pine-apple and highest in oranges (Table 1). Machlin (1991) reported the ascorbic acid content of pine-apple to be 20-40 mg/100 g. The values; 53-55 mg and 27-29 mg/100 g had also been reported for orange and lime juice respectively (The Natural Food Hub, 1999; Vanderslice *et al*., 1990; Oyenuga, 1968; Mathoko and Kiniiya, 2002). The figures reported in this study agreed essentially with these earlier studies but lower in many instances than the values of FAO (1968). The ascorbic acid content of fruits varies appreciably with the fruit maturity, genetic variety, climate and sunlight (Vanderslice and Higgs, 1990; de Ariola *et al*., 1980) and may be responsible for the variation in ascorbate content quoted in literature. The recommended daily intake (RDI) for ascorbic acid - 30 mg/day for healthy women and 40 mg/day for men (NHMRC, 1991) - can be supplied by 100 mL of all the fruit juices investigated except pine-apple.

The constituent organic acids in fruits include ascorbic, malic, lactic, malonic, succinic and citric acids with the latter predominating. In this study, citric acid was highest in lime and lowest in pine apple but generally constituted at least 90% of the total organic acid content of the selected fruits (Table 1).

Total and Available Iron Content: The total iron content presented in Table 2 ranged between 4.2 for lime and 10.7 mg/kg for grape fruit. The iron content of grape fruit reported in this study (Table 2) was higher than the 7 mg/kg reported earlier; that of orange was marginally lower than the 11 mg/kg while the iron content reported for pine-apple agreed with the 4 mg/kg value published by FAO (1968). The total iron content of orange juice was at least twice as high as the 4 mg/kg quoted in Beyond vegetarianism (1994) on the internet. The total iron content of amaranthus and cowpea was 86 and 157 mg/kg respectively. The value for the former sample was lower and that of the latter higher than values previously obtained in our laboratory (Adewusi and Falade, 1996; Adewusi *et al.*, 1999). This is probably a reflection of the soil / location in which these samples were grown.

Percent available iron was high in fruit juices (31-48%) compared to 12% availability of iron for amaranthus and 3% for cowpea (Table 2). The values of iron availability obtained for amaranthus and cowpea are similar to those obtained earlier (Adewusi and Falade, 1996; Adewusi *et al.*, 1999) and close to the 4.1% iron availability reported for green bean (Martinez *et al.*, 1998) and a range of 6-12% iron availability reported by Rangarajan and Kelly (1998) for amaranthus.

The correlation between ascorbic acid and percent iron availability in the fruit juices was negative ($r = -0.80$) probably due to the presence of other organic acids which probably masked its ability as an antioxidant. At high concentration, most anti-oxidants exhibit pro-oxidant activities as observed with ascorbic acid in our laboratory (Falade, unpublished results). Indeed, there was also a negative correlation ($r = -0.52$) between the percent iron availability and the citric acid content of the fruit juices though Hazell and Johnson (1987) had indicated earlier that citric acid enhanced iron availability from wheat flour better than a corresponding concentration of ascorbic acid.

Availability of Iron from Composite Meals: Orange and grape fruit juice each enhanced iron availability in amaranthus by 31 and 10% and in cowpea by 17 and 50% respectively probably due to the presence of higher levels of ascorbic and citric acids. On the other hand, lime and pine-apple juice had only a minor negative (<10%) effect on the iron availability from both amaranthus and cowpea composite diets except the 24% inhibition of iron availability in pine-apple juice-cowpea composite diet. Earlier studies have also revealed the enhancement of iron availability by the addition or presence of ligands such as ascorbic acid (Hazell and Johnson, 1987; Derman *et al.*, 1977), lactic acid (Adewusi *et al.*, 1999) and some amino acids (Reinhold *et al.*, 1981). The observed lack of effect or inhibition of iron availability of iron in pine-apple juice composite diets could be due to the presence of high soluble dietary fiber (0.8%) which is known to bind iron making it unavailable (Fernandez and Phillips, 1982; Falade *et al.,* unpublished results). This inhibitory effect is expected to cancel out the enhancing power of the ascorbic and other organic acids present in the pine-apple juice hence the small values of inhibition mostly observed in both composite diets. Overall, there was high positive correlation ($r = 0.99$) and a significant $r = 0.49$ between citric acid content and iron availability from juice-amaranthus and juice-cowpea composite diets respectively while the correlation between ascorbic acid and iron availability from composite diets was not significant.

Total and Available Magnesium Content: The total magnesium content of the juice from the selected fruits (Table 3) fell within the range of 140-640 mg/kg reported earlier while the value for orange in this report is higher than the content reported earlier (Beyond vegetarianism, 1994). Blanching as a processing method for amaranthus vegetable leaches out minerals, the rate depending on many uncontrollable factors such as the valency of the mineral; whether the metal is bound or free, the binding state and the period of exposure to boiling water. The magnesium value for the present blanched amaranthus sample was lower than the earlier value quoted for a similar sample (Adewusi *et al.*, 1999) but higher than 1024 mg/kg obtained for a sample of amaranthus vegetable obtained from another source (Falade, unpublished results). The total magnesium content of cowpea now reported is similar to 2270 mg/kg reported earlier (Falade and Adewusi, 1996) and within the range observed by Martinez *et al.* (1998).

Percent available magnesium was high in all the fruit juices (Table 3) with no significant correlation ($r = -0.07$) between Mg availability and ascorbic acid but a positive correlation ($r = 0.45$, $p < 0.05$) between availability and citric acid content. The percent available magnesium from amaranthus and cowpea (18 and 23% respectively) were lower than the values obtained for similar samples in earlier studies (Adewusi *et al.*, 1999) and 27-37% reported for green beans by Martinez *et al.* (1998).

Composite Meals: Lime juice produced the highest enhancement of magnesium availability in both amaranthus and cowpea composite diets while pine apple juice enhanced magnesium availability by a marginal 4-5% in both samples (Table 3). The negligible increase in magnesium availability in the presence of pine apple juice may be directly connected to the presence of soluble dietary fiber in the pine apple juice (Falade, unpublished results). There was no significant correlation between ascorbic acid content and available magnesium of the fruit juice-amaranthus composite diet

Table 3: Total and Percent Available Magnesium Content of Some Fruit Juice, Amaranthus Vegetable, Cowpea together with the Theoretical and Experimental Available Magnesium of the Composite Diets[1]

Sample	Total Magnesium mg/kg	% available Magnesium	Available Magnesium (mg/kg) in Fruit-Amarathus and Fruit - Cowpea Composites Diets					
			Fruit Juice + Amaranthus[2]			Fruit Juice + Cowpea[3]		
			Theoretical	Exptal	Df [4]	Theoretical	Exptal	%Df[4]
Pineapple	302 ± 28	36	150 ± 18	157 ± 18	5	181± 13	189 ± 25	4
Orange	220 ± 23	26	106 ± 5	146 ± 17	38	126 ± 25	160 ± 15	27
Grape fruit	155 ± 6.8	53	126 ± 14	169 ± 30	34	162 ± 37	205 ± 61	27
Lime	161 ± 23	39	107 ± 8	159 ± 21	49	143 ± 30	192 ± 28	34
Amaranthus	1356 ± 26	18	-	-	-	-	-	-
Cowpea[5]	2330 ± 3.8	23	-	-	-	-	-	-

[1]Mean ± standard deviation of quadruplicate analysis. [2] Composite diet = fruit juice and amaranthus mixed in ration 4:1. [3]Composite diet = fruit juice and cowpea mixed in ratio 9:1. [4]Df = difference between theoretical and experimental values. [5]Sample was cooked and dried at 50 °C prior to analysis.

Table 4: Total and Percent Available Zinc Content of Some Fruit Juice, Amaranthus Vegetable, Cowpea together with the Theoretical and Experimental Available Zinc of the Composite Diets[1]

Sample	Total Zinc mg/kg	% available Zinc	Available Zinc (mg/kg) in Fruit - Amaranthus and Fruit-Cowpea Composite Diets					
			Fruit Juice + Amaranthus[2]			Fruit Juice + Cowpea[3]		
			Theoretical	Exptal	Df [4]	Theoretical	Exptal	% Df[4]
Pineapple	3.5 ± 0.2	82	6.0 ± 0.3	6.7 ± 0.3	12	6.5 ± 0.2	5.3 ± 0.2	-18
Orange	3.6 ± 0.2	65	5.2 ± 0.01	7.2 ± 0.02	38	6.0 ± 0.1	5.1 ± 0.8	-15
Grape fruit	2.4 ± 0.3	88	6.5 ± 0.1	7.9 ± 0.3	22	7.9 ± 0.4	6.0 ± 0.5	-24
Lime	2.5 ± 0.0	84	7.2 ± 1.8	7.3 ± 2.6	1	7.6 ± 2.3	7.4 ± 0.1	-3
Amaranthus	50.6 ± 0.6	21	-	-	-	-	-	-
Cowpea [5]	67.0 ± 1.0	23	-	-	-	-	-	-

[1]Mean ± standard deviation of quadruplicate analysis. [2]Composite diet = fruit juice and amaranthus mixed in ration 4:1. [3]Composite diet = fruit juice and cowpea mixed in ratio 9:1. [4]Df = difference between theoretical and experimental values. [5]Sample was cooked and dried at 50 °C prior to analysis.

but a negative correlation with juice-cowpea diet (r = -0.40). The correlation between citric acid content and available magnesium of the composite diets were small but positive r = 0.28 and 0.35 fruit juice-amaranthus and -cowpea composite diets respectively.

Total and Available Zinc Content: The total zinc content of the fruit juices presented in Table 4 was in the upper ranges of or greater than 0.03-2.75 mg/kg earlier quoted for fruits (Beyond vegetarianism, 1994) while the zinc content of amaranthus and cowpea was about twice the 24 and 32 mg/kg values reported earlier from our laboratory (Adewusi and Falade, 1996; Adewusi *et al.*, 1999).

Percent available zinc was high (65-88%) in all the fruit juices while the availability from amaranthus and cowpea was about half the 42% availability observed earlier. Numerically however, the figures were identical (Adewusi and Falade, 1996; Adewusi *et al.*, 1999). The correlation factor between ascorbic acid content of the juice and zinc availability was again negative (r = -0.50) but positive for citric acid and zinc availability (r = 0.41).

Composite Meals: Contrary to the effect on magnesium, lime juice did not seem to have any appreciable effect on the enhancement of zinc from both composite diets. Interestingly, the other fruit juice samples inhibited zinc availability from cowpea while enhancing zinc availability by 12-38% in amaranthus composite diets. There is no doubt that the higher level of anti-nutritional factors in cowpea (Adewusi and Falade, 1996) would account for the lower mineral availability and or the no effect syndrome observed with the fruit juices. The inhibition of mineral availability could also be explained if the added ligand increases the level of anti-nutrients that bind minerals. There was a high positive correlation between available zinc and ascorbic acid (r = 0.65) and citric acid (r = 0.99) in the composite diets except in cowpea where r = -0.16 between ascorbic acid and available zinc.

Table 5: Total and Percent Available Copper Content of Some Fruit Juice, Amaranthus Vegetable, Cowpea together with the Theoretical and Experimental Available Copper of the Composite Diets[1]

Sample	Total Copper mg/kg	% available Copper	Available Copper (mg/kg) in Fruit-Amarathus and Fruit-Cowpea Composite Diets					
			Fruit Juice + Amaranthus[2]			Fruit Juice + Cowpea[3]		
			Theoretical	Exptal	Df[4]	Theoretical	Exptal	%Df[4]
Pineapple	0.4 ± 0.04	50	0.9 ± 0.13	0.8± 0.04	-11	1.1 ± 0.13	0.8 ± 0.2	- 27
Orange	0.2 ± 0.04	50	0.8 ± 0.04	0.9± 0.01	13	1.0 ± 0.17	1.1 ± 0.1	10
Grape fruit	0.4 ± 0.02	25	0.8 ± 0.13	0.9± 0.21	13	1.1 ± 0.02	1.3 ± 0.04	18
Lime	0.8 ± 0.13	50	1.2 ± 0.04	0.9± 0.23	-25	1.3 ± 0.05	1.3 ± 0.1	0
Amaranthus	1.1 ± 0.06	36	-	-	-	-	-	-
Cowpea[5]	12.8 ± 2.3	18	-	-	-	-	-	-

[1]Mean ± standard deviation of quadruplicate analysis. [2] Composite diet = fruit juice and amaranthus mixed in ration 4:1. [3]Composite diet = fruit juice and cowpea mixed in ratio 9:1. [4]Df = difference between theoretical and experimental values. [5]Sample was cooked and dried at 50 °C prior to analysis.

Total and Available Copper Content: The total copper content was highest in lime and lowest in orange juice (Table 5). The value for orange was below the 0.5-0.6 mg/kg quoted for fruits (Beyond vegetarianism, 1994) while that of lime in the present study was marginally above it. The copper content of amaranthus reported in this study was in close agreement with that obtained earlier for a similar sample (Falade, unpublished results). The total copper content in cowpea was slightly lower than the 16 mg/kg reported for green beans by Martinez *et al.* (1998) and the 17 mg/kg value observed for a similar sample of cowpea (Falade, unpublished results).

Percent available copper was also high in all the fruit juice samples (25-50%), amaranthus and cowpea (Table 5). As expected, there was no significant correlation either between copper availability and ascorbic acid ($r = -0.35$; $p < 0.05$) or citric acid ($r = -0.08$; $p < 0.05$).

Composite diets: Pine apple and lime juice both impaired copper availability from the two composite diets similar to the observation on the effect of fruit juice on iron availability while orange and grape fruit juice enhanced copper availability by 10 -18%. Ascorbic acid has been reported to inhibit copper availability through the reduction of copper (II) to copper (I) and its subsequent precipitation (Van den Berg *et al.,* 1994) but in this study, there was a positive correlation between copper availability and ascorbic acid ($r = 0.83$ and 0.58) and ($r = 0.49$ and 0.69) in juice-amaranthus and –cowpea composite diets respectively.

Conclusion

This study revealed that the consumption of fruit juice is beneficial to mineral nutriture. Grape fruit and orange juice seem better in this respect than lime and pine apple. It is interesting to note that lime and pine apple with the highest and least citric and total organic acid content affect mineral availability in the same pattern. The mechanism of action would probably be different; the effect of pine apple juice is probably as a result of its low content of anti-oxidant organic acids while the effect of lime juice is presumably due to its large content of organic acids which may act as pro-oxidants at high concentration. Further work with animal models is needed to confirm these results from in vitro studies.

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