

**Amino Acids and Some Minerals in the Nut of the Turkana Doum
Palm (*Hyphaene coriacea*)**



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ABSTRACT

The Turkana doum palm is an important indigenous plant from an economic standpoint in the Turkana District of Kenya. The various parts of the tree are used for a variety of social, cultural and domestic uses with the nut being an important indigenous wild fruit. However, despite the central place it occupies in the diets of those living along the banks of the major rivers, its nutritional profile is lacking in literature.

This study was therefore conducted to document its nutritive composition. In this regard, the protein content of ngapocho and lookot, and the nonessential and essential amino acids in ngapocho were determined. Also, six minerals were determined in the mesocarp products (apinet and ngapocho) of the nut of the Turkana doum palm, *Hyphaene coriacea*. The results showed that ngapocho and lookot contained 1.8 and 4.7% protein, respectively. The mesocarp of the nut contains substantial amounts of both the nonessential and essential amino acids and has a chemical score of 66. Threonine is the limiting amino acid. Unlike most plant foods, the nut is an excellent source of the sulphur amino acids methionine and cystine; it is a good source of isoleucine, leucine, histidine, phenylalanine and tyrosine, and a fair source of tryptophan, lysine, valine and threonine.

Ngapocho/lookot contained 17.0/17.4 mg Ca, 4.8/4.2 mg Mg, 0.2/0.2 mg K, 0.5/0.1 mg Na, 13.9/29.8 mg Fe, and 3.4/4.3 mg Zn/100 g. The nut of the Turkana doum palm is quantitatively an excellent dietary source of iron, a good source of zinc, but is a poor source of calcium, magnesium, potassium and sodium. The addition of bovine blood to apinet to make lookot raises the protein, iron and zinc content by more than 160, 100 and 25%, respectively, thus enhancing the nutritional quality of the lookot. Knowledge of the nutrient composition of indigenous food plants such as the Turkana doum palm is important for the purpose of educating the public on the nutritional value of indigenous food plants available in their immediate environments and for conservation efforts.

Keywords: Amino acids, minerals, Turkana doum palm, Hyphaene coriacea, human nutrition

INTRODUCTION

The palms belong to the family *Palmae* (*Aracaceae*), among which we find the African doum palms [1], represented by the genus *Hyphaene*, the plant of interest in the current study [2]. The African doum palms grow wild in the drier parts of southern, southwestern, north, western and eastern Africa [1, 2]. The doum palms, *Hyphaene compressa* and *H. coriacea*, are important non-cultivated food fruit-plants in the arid and semi-arid districts of Turkana, Samburu and Marsabit of Kenya.

The Turkana doum palm known locally as eengol, grows along the Tirkwell and Keriyo rivers and is an important commercial and food plant in the Turkana District of northwestern Kenya. The epicarp, mesocarp and endocarp make up 9, 28 and 63% of the weight of the fruit on a wet-weight basis (wwb). The mesocarp of the nut is the main edible portion and is processed into three main products; apinet (the coarse ground powder from the sun-dried mesocarp slices), lookot (apinet to which bovine blood is added) and ngapocho (the freshly cut slices of the mesocarp, which may be sun-dried but not ground). Children break the kernel open to expose the edible, oily and coconutty endocarp, which adults rarely consume. All the above food products serve as dry season foods in the zones where the Turkana doum palms grow.

Galvin [3] estimated the protein content of lookot and the semidried doum palm product apinet, but did not determine their amino acids content. There are no reports on the amino acids, magnesium, potassium and zinc content of *Hyphaene compressa* or *H. coriacea* in literature. The protein content of the Turkana doum palm nut was estimated by the Kjeldahl method using a factor of 5.3, as recommended for tree nuts [4]. The metabolic adequacy of protein often referred to as the protein quality, is traditionally assessed in four ways; by the biological value (BV), the net protein utilization method (NPU), protein efficiency ratio (PER) or the chemical score method. The BV, NPU and PER are biological methods which involve the use of animal feeding experiments to estimate protein digestibility and therefore its bioavailability and quality.

The current method for measuring the protein quality of food is the protein digestibility corrected amino acid score (PDCAAS) [5]. It uses the essential amino acid (EAA) content of the test protein, a digestibility evaluation of the protein food, and examines how it meets the requirements of protein for the growth needs of two to five year olds [6]. The above biological methods are both expensive and time consuming unlike the PDCAAS, which is a simpler scoring method based on the limiting amino acid. The traditional chemical, or amino acid scoring method, involves estimating the content of each of the nine EAAs in an experimental food sample and scoring each against the content of the similar amino acid in the standard FAO protein. The standard FAO protein is often egg white or casein, both of which have high digestibility and bioavailability [5, 7] and have a set score of 1.00 or 100%.

The expediency and inexpensiveness of the method makes it ideal for estimating the value of a food as a source of protein for humans. The amino acid with the lowest score or percentage is the limiting amino acid, and is therefore the major contributor to the chemical score of the test protein food [5]. The method, however, does not take into account the digestibility and bioavailability of the protein source under investigation, since animals are not used to test the validity of the score of the food as a source of protein [8]. Nevertheless, the chemical score method was used in the current experiment due to its simplicity.

Chemical and instrumental methods often require preliminary treatment of the test material to hydrolyze the proteins to free amino acids. A major problem with the amino acid analysis in foodstuffs is the destruction of amino acids during acid hydrolysis. Unfortunately, this problem can be greatest with the EAAs methionine, cystine, lysine, threonine and tryptophan [9], which are likely to be limiting in practical diets [10]. Proteins and protein foodstuffs differ so widely in their composition that "ideal" hydrolytic procedures would need to be almost specific for each material. Thus, compromises between the ideal and practical are often necessary [11].

Assessment of amino acid composition has been done using three or four acid hydrolyses of different time durations (usually 24, 48, and 72 or 96 h). Five or six separate hydrolyses are recommended for accuracy. A special acid hydrolysis following performic acid oxidation to obtain accurate values for cysteine acid and methionine sulphone, and an alkaline hydrolysis to obtain an accurate value for tryptophan should also be performed [11]. The four hydrolysis times are designed to allow selection of specific times to obtain the best results for each amino acid, e.g., threonine and serine from extrapolation to zero time, tyrosine after 24 h, isoleucine and valine values after 48 or 72 h, and the remaining amino acids as the mean of the four determinations [11]. Separate procedures for the sulphur amino acids and for tryptophan are always essential; however, in most cases, a single 24-h acid hydrolysis can give adequate information for scoring purposes when cost is an important consideration. In acid hydrolysis, glutamine and asparagine are converted to glutamic and aspartic acids, respectively, and their amounts are therefore included in the totals of glutamic and aspartic acids, respectively.

For tryptophan, high-performance liquid chromatography (HPLC) can be used to estimate its amount in a food sample. Post or pre-column derivatization using dansyl chloride or another derivatizing agent may be necessary to obtain a form that can be determined by either UV or fluorescence detectors [12]. In the reversed phase HPLC procedure, the common carrier phases include mixtures of acetonitrile and water, and methanol and water. An internal standard of norleucine is commonly used [12]. Pure tryptophan was used in the current work for the construction of a standard curve used to estimate the amount of tryptophan in the ngapocho by reversed phase HPLC.

As far as human nutrition is concerned, the inorganic nutrients currently considered crucial for life include water, sodium, potassium, chloride, calcium, phosphate, sulfate, magnesium, iron, copper, zinc, manganese, iodine, selenium, and molybdenum [13]. The minerals measured in this study by absorption spectrophotometry are sodium, potassium, calcium, magnesium, iron, and zinc. While the wild fruit eengol is an important dietary item for the Turkana people of Kenya, especially in the dry season, no study has been undertaken to provide the nutritional composition of the fruit. This study although not extensive due to cost considerations, was therefore performed to determine the amino acids of ngapocho and the protein and mineral profile of ngapocho and lookot, two main food products traditionally made from eengol.

2.0 Materials and Methods

Ready-to-eat apinet, lookot and whole sun-dried ripe fruits of *Hyphaene coriacea* were shipped from Kenya to the Department of Food Science, University of Reading, Reading, U.K. The materials had been bought ten days earlier in the open-air market in the town of Lodwarr, Turkana District, Kenya, and were stored at -10°C in the Department of Dairy and Food Science and Technology, Egerton University, Kenya. In the U.K. the samples were stored at -20°C until analyzed. All chemicals used were analytical grade (Fisher Scientific, Cambridge, U.K.) unless otherwise stated.

2.1 Protein estimation by Kjeldahl Method

The protein content of samples were determined following AOAC [14] Method 981.10 using duplicate samples (1.0 g) each of lookot and ngapocho. Copper sulphate was used as the catalyst. The crude protein was calculated using a conversion factor of 5.3 [4]. The non-protein nitrogen (NPN) value, which was determined using 5% trichloroacetic acid (TCA) [15], was subtracted from the crude protein to give an estimate of the protein nitrogen. The determinations were done in duplicate and the mean values are reported.

2.1.1 Determination of all amino acids except tryptophan, cystine and methionine

Duplicate samples (0.1 g) of the ground ngapocho were transferred into two hydrolysis tubes and 20 mL 6M HCl added following AOAC [16] Method 982.30. The tubes were evacuated and flushed with nitrogen, and the tubes sealed using a rubber ring; the contents were heated in an oven (LKB 4400, Biochrom LKB, St Albans, U.K.) to 110 °C and maintained at this temperature for 24 and 48 h. After hydrolysis, the contents were cooled, transferred quantitatively to 500-mL volumetric flasks, and the volume made up to the mark with HPLC grade double distilled water. The mixture was then filtered through a Whatman filter paper No. 541.

Ten mL aliquots were pipetted into 100-mL round-bottomed quick-fit flasks and evaporated to dryness at 50°C under vacuum to remove the acid. Five mL of distilled deionized water was added and the samples evaporated to dryness again. The sample residue was then dissolved in 0.2M sodium citrate buffer of pH 2.2, stored in McCartney bottles, and frozen at -30°C until analyzed using the amino acid analyzer (SP9, Pye Unicam, San Jose, California, U.S.A.) fitted with a spectrophysics SP 4100 integrator (CarloErba Instrumentazione, Milan, Italy).

Ninhydrin was used for amino acid detection and nitrogen as the carrier gas; the ion exchange column was a Model No. 26017100 unit (CarloErba Instrumentazione, Milan). Results obtained from the 24-h acid hydrolysis were extrapolated to time zero for all amino acids, except cystine, valine, leucine, methionine, isoleucine, and tryptophan. The amounts of valine, leucine and isoleucine were obtained from the 48-h hydrolysis by extrapolation to time zero.

2.1.2 Determination of cystine and methionine

Duplicate samples (0.1 g) were weighed into round-bottomed flasks and 10 mL of 80% performic acid added following the procedure of Strydom [17]. The mixture was made up to 100 mL with deionized water and left overnight in ice. The following morning, 1.5 mL hydrobromic acid was added and shaken before reduction of the volume about 50 mL under vacuum.

This was followed by the addition of 30 mL 6M HCl, heating the mixture in the oven (step 2.1.1) to 110°C and maintaining it at this temperature for 24 h. The hydrolysate was filtered, washed with distilled water and made to 50 mL. Five mL of the filtrate was evaporated under vacuum and 10 mL of sodium citrate buffer pH 2.2 was added and diluted five times before aliquots were loaded into the amino acid analyzer. Norleucine was the internal standard while ninhydrin was used for amino acid detection.

2.1.3 Determination of tryptophan

Sixteen mL of 4.3M lithium hydroxide containing 1% (w/v) thiodiglycol was measured into thick-walled glass hydrolysis tubes containing duplicate 0.1 g sample of ngapocho following the procedure of Strydom [17]. Hydrolysis was carried out at 110°C for 16 hr. The hydrolysate was neutralized with 4.0M perchloric acid and adjusted to pH 3.0 with acetic acid, cooled, filtered, and diluted to 50-mL volume with distilled water in a volumetric flask.

The samples were diluted 10x before injecting into a reversed phase HPLC (Model 3A29, CarloErba Instrumentazione, Milan); the stationary phase was Zorbax C₈, in a 25 cm, 4.6 mm x 2.5 mm i.d. column (Scientific Optical Solutions, Glasgow, U.K.). Tryptophan was detected at 220 nm by a variable wavelength detector (Model 110, Knauer GmbH, Berlin, Germany) using HPLC-grade methanol and water (40:60, v/v) as the mobile phase at a flow rate of 1.0 mL/min.

The amount of tryptophan in the aliquot was determined from the area under its peak and the corresponding concentration from a standard curve made using 0.1, 0.2, 0.5, 1.0, 1.5, 2.0 and 4.0 ppm HPLC-grade pure tryptophan run under identical experimental conditions.

2.2 Determination of minerals in ngapocho and lookot

2.2.1 Ashing (AOAC Method 940.26A) [14]

Ten gramme duplicate samples of ngapocho were weighed into a porcelain dish and ashed at 525°C for 1 h. The charred mass was treated with water to dissolve soluble solids. It was filtered through ashless paper and both the residue and paper were ignited to white ash. The filtrate of soluble salts was added, evaporated to dryness and again ignited at 525°C to constant weight. The mass was transferred quantitatively to a previously oven-dried 50-mL volumetric flask, tightly stoppered and stored in a dry place until required for step 2.2.2.

2.2.2 Estimation of minerals in ash (AOAC Method 968.08) [14]

Standard solutions of sodium, potassium, calcium, magnesium, iron and zinc were made from NaCl, KCl, CaCl₂, MgCl₂, granules of iron and zinc, each made into a stock solution of 1000 ppm of the cation. A standard curve for each mineral was constructed from dilutions of 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 20.0, 40.0 ppm made from the stock solutions; each dilution of the respective minerals was aspirated into an Atomic Absorption Spectrophotometer (AAS).

The corresponding readout signal was noted and used to plot the standard curves. Strontium chloride was added to the standards and experimental samples to reduce interference from proteins, phosphates and sulphates. The dissolved ash from step 2.2.1 was aspirated into the AAS and the amount of mineral in the sub-sample corresponding to the integrator readout was determined from the respective standard curve. The estimation of each mineral in solution was done in triplicate.

Results

Ngapocho and lookot contained 1.8 and 4.7% protein, respectively. The mesocarp of the nut of the Turkana doum palm is an excellent source of the sulphur amino acids methionine and cystine; it is a good source of isoleucine, leucine, histidine, phenylalanine and tyrosine, and a fair source of tryptophan, lysine, valine and threonine. It has a chemical score of 66, with threonine being the limiting amino acid. The ngapocho/lookot contained 17.0/17.4 mg calcium, 4.8/4.2 mg magnesium, 0.2/0.2 mg potassium, 0.5/0.1 mg sodium, 13.9/29.8 iron, and 3.4/4.3 mg/100 g zinc.

Discussion

Ngapocho and lookot contained 1.8 and 4.7% protein, respectively. The low protein content of the two food products (Table 1), may not alleviate malnutrition related problems in adult consumers in this community with about 81% of the population with insufficient food and 74% living below the absolute poverty level [18]. Galvin

[3] estimated the crude protein content of the dried apinet (4% moisture content) as 3.8%, which compares with the 1.8% in the semi-dry slices of ngapocho with 23% moisture. Besides the difference in the moisture content of the two products, inherent variability in natural products and differences arising from experimental variability may partly explain the apparent compositional differences.

Despite the low protein content, the mesocarp of the Turkana doum palm nut has a high content of methionine and cystine (Table 2). Methionine and cystine are generally low in many plant materials including some nuts [19]. A high sulphur content of the soil where the plants grow may contribute to the high sulphur amino acids content in the nut [13], although the sulphur content of the riverine soils in Turkana was not available at the time this study was being done. Compared to the FAO/WHO ideal protein, and similar to many plant food materials, the nut is high in leucine, isoleucine, and phenylalanine and tyrosine (Table 3).

However, it is low in valine, lysine, threonine and tryptophan, and has a chemical score of 66. Threonine is the limiting amino acid. The relative lack of lysine is widespread in large areas of the world predominantly consuming cereal-based diets, including parts of Africa [20]. The problems posed by the low amounts of lysine in diets can be exacerbated by its tendency to participate in chemical reactions including the maillard reaction with reducing sugars during high temperature food processing and prolonged storage [21,22]; apinet and the whole fruits are sun-dried at an ambient air temperature prevailing in Turkana of 40-45°C, for as long as seven days, and can be stored for up to four months at 30-38°C before consumption [23].

Although the age from the time of harvest of the nuts, the ngapocho and lookot was not known with certainty, it was estimated to be about 2 months at the time of purchase. The nuts and its two food products were stored at -20°C for a further 3 months before they were analyzed. The effect of the processing and storage procedure on the amino acid content was not, however, verified. Lysine also cross-links with the side chains of dehydroalanine, serine and cysteine under alkaline conditions, leading to further losses and reduced access by proteolytic enzymes [19].

Tryptophan is used for the synthesis of haemoglobin and plasma proteins and is low in maize diets [24], which are staple foods in most African communities in Kenya, including the urbanized Turkana people. The human body can make niacin from tryptophan, although only small amounts can be made in this way since it is an inefficient process, as only 0.1 g niacin can be made from 6 g tryptophan [24]. From the work of Galvin [3], the apinet of the Turkana doum palm nut has a niacin content of 3.4 mg/100 g, which is comparable to 5.0 and 4.7 mg/100 g in wheat flour and brown rice, respectively [19]. However, its high dietary fibre content of 27% [23] may reduce niacin bioavailability, since niacin tends to bind to hemicellulose in high-fibre plant foods [19, 24]. Overall, ngapocho is a fairly good source of the EAAs, despite being a poor protein source.

Where mixed diets are consumed in adequate quantity, it is likely that the low protein in the nut products will be complemented by animal foods such as fish or livestock meat; these are locally available high protein foods with high biological value proteins [24]. Addition of bovine blood to apinet to make lookot enhances its protein content and possibly its amino acid profile.

Both ngapocho and lookot are quantitatively excellent sources of iron as they contained 13.9 and 28.9 mg/100 g, respectively (Table 4). The higher iron content in lookot is due to the addition of bovine blood to apinet. Earlier reports indicate that the riverine communities that subsist on eengol can consume up to 500 and 100 g/day of ngapocho and lookot, respectively, during the harvest period [23]. Since the RDA for iron is 8 mg/day for young adults of both sexes as well as adults older than 50 years [8], it is likely that the consumption of the indicated quantities of the nut products can supply the RDA of iron for these groups of consumers over the time.

However, the high dietary fibre content of 27% in ngapocho [23] is likely to reduce iron absorption [13]. It is also generally recognized that non-haeme iron from plant foods is absorbed in lower quantities as compared to haeme iron from animal meats [13]. The nut is a fairly good dietary source of zinc, whose content rises with the addition of bovine blood to apinet. It contains 3.4 and 4.3 mg/100 g in ngapocho and lookot, respectively (Table 4). For adult consumers of both sexes, about 300 g of both food products can supply the RDA of 11 mg/day [8]. This amount appears to be within the amount of ngapocho (the most consumed commodity) consumed daily by some members of the local population during the season when the ripe fruits are harvested according to earlier findings [23]. The most prevalent micronutrient deficiency in the world is lack of iron (over 2,000 million people affected), while a lack of zinc, selenium, and other trace elements also affects large numbers of people in various parts of the World [25]. It is likely that some residents of Turkana may be shielded from the micronutrient deficiencies related to iron and zinc metabolism when they subsist on eengol and its products, and more so the lookot in which bovine blood is an ingredient.

Ngapocho and lookot are poor dietary source of calcium, magnesium, potassium and sodium, although lookot is a better source of potassium than ngapocho. While milk and dairy products are excellent sources of calcium (119-125 mg/100 g), the two food products from the Turkana doum palm nut have a calcium content (17-18 mg/100 g) that is higher than in dried maize meal (6 mg/100 g), lean beef with a 55-57% moisture content (9-13 mg/100 g), the potato (10 mg/100 g) and rice (4 mg/100 g). The calcium content of ngapocho from *Hyphaene coriacea* is lower than that in *Hyphaene compressa* as determined by Hoebekke [26], probably due to species differences, methodology, sample variability and/or differences in proximate composition. Both doum plant species are found in Kenya. Since both sodium and potassium are recirculated according to body needs, deficiencies from these two minerals are rare since they are found in every food [27].

Cases of rickets in the young and osteoporosis in the elderly cannot be ruled out in the relevant parts of Turkana District as being contributed by considerable consumption of eengol and lack of adequate intake of milk and dairy products.

Knowledge of the nutrient composition of the Turkana doum palm is crucial for the purpose of educating the public on the nutritional value of indigenous food plants available in their immediate environments. Knowing the nutrient content of indigenous food plants such as the Turkana doum palm can assist conservation efforts of wild food plants some of which are threatened with extinction.

Conclusions

Ngapocho and lookot contained 1.8 and 4.7% protein, respectively. The nut contains substantial amounts of both the nonessential and EAAs and has a chemical score of 66. Threonine is the limiting amino acid. The nut is an excellent source of methionine and cystine, a good source of isoleucine, leucine, histidine, phenylalanine and tyrosine, and a fair source of tryptophan, lysine, valine and threonine.

The ngapocho/lookot contained 17.0/17.4 mg calcium, 4.8/4.2 mg magnesium, 0.2/0.2 mg potassium, 0.5/0.1 mg sodium, 13.9/29.8 mg iron, and 3.4/4.3 mg/100 g zinc. Ngapocho is an excellent dietary source of iron, a good source of zinc, but is a poor source of calcium, magnesium, potassium and sodium. Addition of bovine blood to apinet to make lookot, raises the protein, iron and zinc content by more than 160, 100 and 25%, respectively, thus enhancing the nutritional quality of the apinet and ngapocho. It is a prudent procedure that needs to be encouraged.

Despite the apparently good amino acid and mineral profile, there is need to do more research work on the antinutrients in the edible portions of the nut to establish more clearly its likely contribution to the diets of the residents of the zones where the palms grow.

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Table 1-The protein content of lookot and ngapocho (g/100 g food)

Property	Ngapocho	Lookot
Non-protein nitrogen	0.05	0.03
Crude protein	1.82±0.6	4.76±0.9
Protein nitrogen	1.8	4.7

n = 4 for both protein and moisture estimation; moisture content of ngapocho and lookot was 23.0 and 16.5%, respectively (wet weight basis, ww).

Table 2-The content of essential amino acids (mg/g protein) in hen's egg and ngapocho

Amino acid	Hen's egg	Ngapocho	Amino acid score
His		41	
Ile	54	51	94
Leu	86	77	90
Lys	70	51	73
Met+Cys	57	197	346
Phe+Tyr	93	96	103
Thr	47	31	66
Trp	17	14	82
Val	66	45	69

Source for hen's egg amino acid content [7]

Legend: His-histidine; Ile-isoleucine; Leu-leucine; Lys-lysine; met-methionine; Cys-cystine; Phe-phenylalanine; Tyr-tyrosine; Thr-threonine; Trp-tryptophan; Val-valine.

Table 3- Amino acid content of ngapocho and FAO/WHO "ideal protein" (mg/g protein) and adult daily amino acid requirements (mg/Kg/day)

Amino acid	Amino acid amount in ngapocho (mg/g protein) (% of FAO/WHO "ideal protein")	Adult (18+ years) amino acid requirement (mg/kg/day)	Amino acid content (mg/g) in FAO/WHO "ideal protein"
His	14.6±2.8 (91)	8-10	16
Ile	13.0±2.0 (100)	10	13
Leu	20.7± 3.6(109)	14	19
Lys	11.5±2.4 (72)	12	16
Met+Cys	33.3±4.1 (196)	13	17
Phe+Tyr	21.9±3.9 (115)	14	19
Thr	5.2±0.7 (58)	7	9
Trp	4.1±0.6 (82)	3.5	5
Val	7.6±0.8 (59)	10	13
Arg	16.1±3.0	-	-
Asa	18.8±6.9	-	-
Ser	14.6±1.2	-	-
Glu	54.1±6.3	-	-
Pro	19.9±2.1	-	-
Gly	13.2±4.6	-	-
Ala	12.8±2.0	-	-

n = 2 for amino acid estimation; source of adult human amino acid requirements [20]. Legend: Arg-arginine; Asa-aspartic acid; Ser-serine; Glu-glutamic acid; Pro-proline; Gly-glycine; Ala-alanine.

Table 4-The mineral content of lookot and ngapocho (mg/100 g food product)

Mineral	Mineral content in ngapocho	Mineral content in lookot
Calcium	17.0±2.9	17.43±1.6
Magnesium	4.8±0.7	4.15±1.1
Potassium	0.19±0.03	0.24±0.01
Sodium	0.47±0.1	0.12± 0.04
Iron	13.9±1.8	29.8±3.9
Zinc	3.40± 0.8	4.29±0.9

n = 2

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