

Quantitative Analysis of Selenium in Staple Foodstuffs from Kanam Local Government Area, North-Central Nigeria

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Abstract

Background: Although selenium was, for decades, considered toxic, many animal diseases has been found to respond to selenium. Selenium is, therefore, an essential trace element. Selenium is essential for human nutrition where it is a constituent of more than two dozen selenoproteins that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection. Selenium concentrations in plant-based foods vary widely by geographic location. This research was aimed at determining the amount of selenium in the local foodstuffs from Kanam Local Government Area in north-central Nigeria.

Methods: Staple foodstuffs were selected across all the sections of the research area. Samples were homogenized in a ceramic mortar to obtain homogeneous samples with particle size > 300µm. These were digested using a diluted oxidant mixture containing 1 ml deionised H₂O + 0.5 ml double-distilled HNO₃. Selenium content was determined using the inductively coupled-mass spectrophotometry technique. Data obtained were analysed using the student's t-test and results were presented as means and standard deviations. A probability level of $p < 0.05$ was considered statistically significant.

Results: All the foodstuffs contained quantities of selenium high enough to meet the recommended daily allowance for all categories. Grain varied widely in their selenium content with white sorghum containing the lowest (6.16 ± 3.97 µg/g) and red sorghum containing the highest (14.19 ± 7.22 µg/g) followed by cowpea (13.05 ± 8.79 µg/g).

Conclusion: These values suggest that a diet well balanced in other nutrients is probably also nutritionally adequate with regard to selenium, although possible effects of cooking, processing, geographical variation and biological availability remain to be investigated.

Key Words: Staple Grains; Kanam; Selenium; North-Central Nigeria

Introduction

According to Oldfield, selenium (Se) was discovered in 1817 by Jacob Berzelius when investigating the chemicals responsible for outbreaks of ill health among workers in a Swedish sulphuric acid plant [1]. It was, later on, shown that selenium prevents liver necrosis in rats [2]. Despite earlier concerns for selenium toxicity, certain animal diseases have been found to respond to

selenium [3, 4]. Selenium is, therefore, an essential trace element occurring in organic (selenomethionine and selenocysteine) and inorganic (selenate and selenite) forms. The organic form is found predominantly in grains, fish, meat, poultry, eggs and dairy products, and enters the food chain via plant consumption. Selenium is essential for human nutrition where it is a constituent of more than two dozen selenoproteins (such as glutathione peroxidase and selenoprotein P) that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis and protection from oxidative damage and infection [5]. For instance, selenium improved glutathione peroxidase and CD4 count in HIV positive Nigerian patients [6-8]. Selenium is also found to exert a positive influence on male reproductive function and accelerated gastric ulcer healing in an animal study [9]. The mineral has also been shown to possess insulinotropic antioxidant, and anticancer effects [10-12]. Selenium, among other functions, reduced susceptibility to infection in rats [13].

Selenium deficiency has been associated with a number of diseases including Keshan disease, a myocardial disease with necrotic lesions, among others. Clinically, Keshan disease showed acute or chronic episode of a heart disease characterized by cardiogenic shock, enlarged heart, congestive heart failure, and irregular heartbeat (arrhythmia) [15].

In terms of distribution, there is marked geographic variability of Se in food related to local soil content [16]. The amount of selenium in a given type of plant-based food depends on the amount of selenium in the soil (and other factors, such as soil pH, amount of organic matter in the soil), and whether the selenium is in a form that is capable of being taken up by plant [11, 17]. Although the selenium content of a wide variety of foodstuffs has been surveyed in the West African Food Composition Table and in other literatures, there exist few data concerning amounts of selenium in foods consumed by Nigeria [18]. Therefore, this research was aimed at determining the amount of selenium in the local foodstuffs from Kanam Local Government Area in north-central Nigeria with a view to establishing the daily dietary intake of selenium of the populace and comparing with recommended dietary allowance.

Materials and Methods

Samples

Foods samples representing a cross section of the north-central Nigerian diet were chosen for analysis. Samples were obtained from farmlands in Kanam Local Government Area of Plateau State, Nigeria. Staple foodstuffs were selected in all cases. All samples were assayed as obtained from farmlands with no cooking or drying performed, since selenium might be lost as a result of these processes.

Preparation of samples

Samples were homogenized in a ceramic mortar to obtain homogeneous samples. Only edible portions were used with the husks being discarded in most cases (particle size > 300µm). Sample masses ranging from 0.192 to 0.223g (average 0.208g) were weighed on Denver Instrument M – 310 (Aldinger Company, USA) weighing balance. All sample handling was performed wearing disposable, powder-free, latex gloves in clean areas. A combination of wet and dry ashing technique, adapted (with modification) from Hill et, al was used in the preparation of the food samples [19].

These were digested using a diluted oxidant mixture (1 ml deionised H₂O + 0.5 ml double-distilled HNO₃). The samples, in 13 x 100mm borosilicate tubes were then placed in a heating block (Isotemp Dry Bath 145, Fisher Scientific Inc., Bohemia, NY, USA) and hydrogen peroxide (0.5 ml) was added to each to complete the first step of the digestion process. The operating temperature for this step was 95oC. At the end of this initial digestion, almost all the samples gave a black mass residue. The tubes are then placed upright in inverted 1000 ml glass beaker, covered with a watch glass, and placed in a muffled furnace (Ashing Oven Lindberg, USA).

Furnace temperature was set to increase at 50oC/h from an initial 100oC, to 375oC. The temperature was held at 375oC for 48 h. Thereafter, it decreased at the same rate as it was increased until it reached room temperature. After cooling, samples were removed from the furnace and 0.20 ml (i.e. 200 µl) of deionised water [obtained from a Milli – Q water purification system (Millipore, Belford, MA, USA)] and 0.20 ml (200 µl) of double-distilled nitric acid (GFS Chemicals, Inc., Columbus, OH, USA) were added to each.

Tubes were, again, placed in heating blocks (Isotemp Dry Bath 145, Fisher Scientific Inc., Bohemia, NY, USA) and the temperature was raised to 90oC first and then, 95oC. Hydrogen peroxide (50%) was added in 0.10ml (100µl) aliquots at 10 -15 minutes interval until all black carbon particles were digested. Samples were allowed to evaporate to dryness and cooled. A white ash was obtained from each sample and 1.8ml of deionised water and 0.2ml of 1% double–distilled nitric acid were added to each (presumably one could use HCl in situations where nitrate ion is undesirable). Samples were reheated at 90oC for 15 minutes to dissolve the residue (ash) and cooled. From this solution, 0.1ml

(100µl) was taken in 10ml polypropylene tubes and made up to 5.0ml by adding the following:

- 0.01ml (10µl) of 1:10 gallium, Ga, solution as internal standard;
- 4.89ml of 0.1% double-distilled nitric acid.

Each tube was shaken on a Vortex (Vortex Genie 2TM, Fisher Scientific Inc., Bohemia, NY, USA) and then, immediately used for the ICP-MS.

Instrumentation procedure

Selenium content was determined using the inductively coupled-mass spectrophotometry technique on a Perkin Elmer SCIEX™ ELAN® 9000 ICP-MS (Norwalk, CT, U.S.A.).

Internal standardisation with gallium in 2% HNO₃ (Perkin Elmer Life and Analytical Sciences, Shelton, CT, U.S.A.) was performed at 0.1 µg/l concentrations to all measuring solutions for the correction of matrix effects and quantification by external standardisation with standards prepared by serial dilution of 1g/l multi-element standard [(Perkin Elmer Life and Analytical Sciences, Shelton, CT, U.S.A.) Choi et, al [20]. Working standards for Se were freshly prepared by diluting 1000 ppm pure Se stock solution (PerkinElmer) in a solution of 0.1 % HNO₃. The ICP-MS was operated at 1,400 W forward power with a coolant flow rate of 13.5 l/min, nebulizer gas flow rate of 1.15 l/min with concentric nebulizer. Spray chamber temperature was 4oC with cyclonic chamber. Sample delay and rinse times were 45 s with single reading. Sample uptake rate was 40 rpm.

Statistical analysis

Statistical analysis of data was performed using SPSS for Windows [(v17) SPSS Inc., Chicago]. The statistical programme was SPSS Statistics Data Editor. Data were presented as means and standard deviations. The student's t-test was used to determine the selenium content of grains and nuts in the region. Results are expressed as arithmetic means ± standard deviation-SD. A probability level of P < 0.05 was considered statistically significant.

Results

Most food samples were found to contain less than 7µg Se/gramme as shown in Table 1.

Some of the samples contained considerably higher values ranging from 9.03±6.05 µg /g for *Pennisetum glaucum* (millet) to 14.19µg/g for red *Sorghum bicolor* (sorghum). It is interesting that red sorghum had the highest selenium content of the food samples assayed despite not having the garlic-like smell reported for certain selenium compounds (Figure 1) [2].

As can be seen from Table 2, there is, for each crop, a wide variation in selenium content. Both in terms of lowest and highest selenium level detected and the mean values, red sorghum (*Sorghum bicolor*) has the highest followed by cowpea (*Phaseolus vulgaris*), and groundnut (*Arachis hypogea*), while white sorghum (*Sorghum bicolor*) has the lowest selenium content followed

Table 1: Some Human selenoproteins*

Selenoprotein	Function	Localization
15kDa Selenoprotein	Gatekeepers for quality control by glucosylating misfolded proteins, thereby preventing transport of improperly folded glycoprotein's out of the ER.	Localized in the ER; mainly expressed in the prostatic gland, testes, brain, kidney and liver, low levels also Detected in skeletal muscle, mammary gland and trachea.
Deiodinases (DIO)	Cleave specific iodine carbon bonds in thyroid Hormones thereby regulating their activity.	Thyroid gland, anterior pituitary of the brain.
Deiodinase 1 DIO1	Primarily deiodinates the 5' -position of the phenolic Ring, but can also deiodinate the 5-position.	Homodimeric plasma membrane protein.
Deiodinase 2 DIO2	Deiodinates the 5'-position with a preference for T4 over rT3.	Central nervous system, pituitary and thyroid glands, skeletal and heart muscle, and in placental and brown adipose tissue.
Deiodinase 3 DIO3	Deiodinates the 5-position of the tyrosyl ring.	Brain, placenta and pregnant uterus.
	Inactivation of T3 and T4.	
Glutathione Peroxidases	Reduce and thereby detoxify peroxides to their respective alcohols at the expense of (typically) glutathione.	Ubiquitous homotetrameric cytosolic enzymes.
Glutathione peroxidase 1 GPx1	Protect the cell from apoptosis, involved in the regulation of virus production.	Ubiquitous homotetrameric cytosolic enzyme (often referred to as cGPx). GPx1 is abundant in the liver and erythrocytes.
Glutathione peroxidase 2 GPx2	Accepts organic hydroperoxides (but not phosphatidyl choline hydroperoxide) as substrates. GPx2 could be involved in apoptosis and proliferation.	Liver and within the gastrointestinal system (but absent in heart and kidney). GPx2 is oftenreferred to as GI-GPx.
Glutathione peroxidase 3 GPx3	Not convincingly resolved. So far, an efficient reductant. May have regulatory functions. GPx3 is primarily expressed in the renal proximal tubules and is used as a marker to monitor tubular integrity	Located extracellularly in the plasma, hence the acronym pGPx, and in the intestine. GPx3 has the second highest plasma concentration after selenoprotein P.
Glutathione peroxidase 4 GPx4	Exhibits the broadest substrate specificity of all glutathione peroxidases and can even reduce phospholipid hydroperoxides (often referred to as ph-GPx), as a universal antioxidant in the protection of biomembranes. GPx4 is also involved in redox signaling and regulatory processes, such as inhibiting lipoxygenases and apoptosis.Antioxidative function.	Testes, where it accounts for almost the total selenium content.
Glutathione peroxidase 5 GPx5	Backup for the selenocysteine-containing isoforms in sperm.	Found exclusively in the epididymis. Only in olfactory epithelium and embryonic tissues
Glutathione peroxidase 6 GPx6	Suggests a function for GPx6 in olfaction.	
Glutathione peroxidase 7 GPx7	Involved in breast cancer cell defense against oxidative stress generated from polyunsaturated fatty acid metabolism.	Mammary gland.

Selenoproteins H, I, K, N, O, P, R, S, T, V, W.	Redox active proteins; heavy metals antidotes. Involved in the retrotransport of misfolded luminal ER proteins to the cytosol for proteasome degradation in a ubiquitin-dependent manner.	Expressed in many tissues: liver, transcripts are detected in skeletal muscle, brain, lung and placenta.
Selenophosphate Synthetase 2	Required for the formation of tRNA [Ser] Sec-bound selenocysteine.	All tissues.
Thioredoxin Reductase Family	Involved in a myriad of cellular and intercellular Processes.	All mammalian tissues.
Thioredoxin Glutathione Reductase	Specific function is currently unknown; it can reduce glutathione disulfide.	Is a testis-specific enzyme.

* Extracted from Gromer et al. [14]

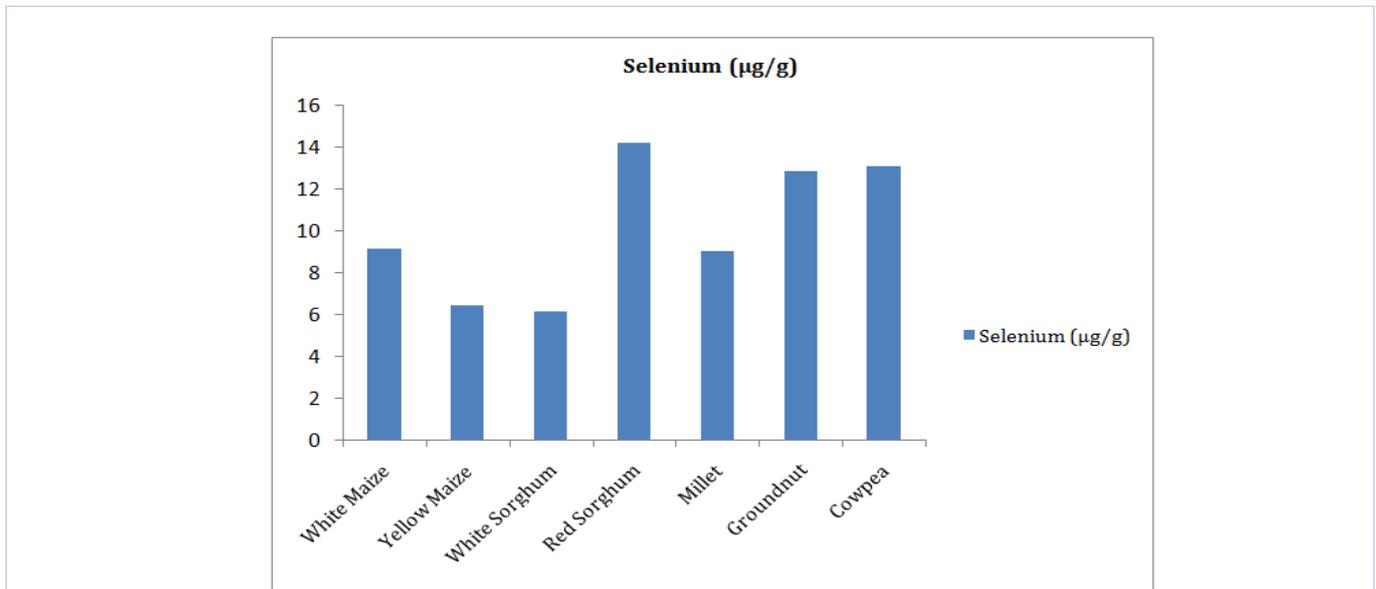


Figure 1: Graphical representation of selenium in the staple grains

Table 2*: Selenium content (µg/g dry weight) of staple grains in various parts of the study area

Sample	1	2	3	4	5	6	7	8	9	L.G.A. Mean
White Maize	12.13	8.10	4.6	13.53	3.43	6.49	17.83	6.05	10.21	9.15±4.69
Yellow Maize	6.02	1.45	5.49	2.72	4.41	8.4	15.73	5.83	8.03	6.45±4.14
White Sorghum	1.11	14.74	6.39	10.01	7.99	8.02	6.11	3.54	4.27	6.16±3.97
Red Sorghum	10.93	9.94	24.66	16.41	24.79	5.25	11.8	17.68	6.22	14.19±7.22
Millet	2.93	6.76	3.84	15.37	20.3	7.89	2.37	12.31	9.47	9.03±6.05
Groundnut	1.9	13.63	34.14	9.12	11.51	9.12	14.58	5.5	15.92	12.82±9.15
Cowpea	11.86	0.95	29.87	15.03	19.51	10.18	13.86	1.62	14.57	13.05±8.79

*Tabulated data are means ± SD of 3 determinations for each sampling area

Table 3* : Recommended Dietary Allowance (RDA) for Selenium

Life Stage	Age	Males (µg/day)	Females (µg/day)
Infants	0-6 months	15 (AI)	15 (AI)
Infants	7-12 months	20 (AI)	20 (AI)
Children	1-3 years	20	20
Children	4-8 years	30	30
Children	9-13 years	40	40
Adolescents	14-18 years	55	55
Adults	19 years and older	55	55
Pregnancy	all ages	-	60
Breast-feeding	all ages	-	70

*Adopted from: Food and Nutrition Board, Institute of Medicine [21]; AI = Adequate Intake

by yellow maize (*Zea mays*). In each case, the difference is statistically significant ($P < 0.05$). So, among the staple foodstuffs of the areas sampled, red sorghum and cowpea appear to be the richest source of dietary selenium followed by groundnut while white sorghum and yellow maize are the poorest, in that order.

Compared to the recommended dietary allowance of selenium, the samples analysed here have high quantities of selenium enough to meet the RDA Table 3.

Discussion

The present paper reports values for selenium in a variety of common food items assayed using an inductively coupled-plasma mass spectrophotometric technique. This technique allows the precise and accurate determination of selenium in the nanogramme range. The values found suggest that, with certain qualifications, there exists an adequate level of selenium in the human diet of north-central Nigeria. Selenium intake of humans ranges between very wide limits due to the consumption of foods with different selenium content. Combs showed in his study that in the different parts of the world there can be a difference of orders of magnitude in the selenium intake of people [22]. In China for example in the Keshan area the selenium intake varies between 7-11 µg/day, whereas in the Enshi county of central China it can reach several thousand µg per day. In Europe selenium consumption of adults is around 30-100 µg per day, in North America 60-220 µg/ and in New Zealand's selenium deficient areas in some populations the daily selenium intake ranges between 19-80 µg/day. In some European countries selenium intake has decreased significantly in recent decades [23]. The main reason for this is the decreased import of bread making wheat from North America which contains generally much more selenium than the wheat grown in the UK [24].

The present results suggest that the levels of selenium in Nigerian staple foodstuffs (at least from the area studied) are sufficient for good nutrition. While this work was in progress, a review concerning selenium micronutrient appeared in Nigeria: the review reported results that generally agree with our data

when expressed on an equivalent basis [25]. Thompson and Scott have shown earlier that 0.04 to 0.10 ppm selenium are needed in the diet to prevent selenium deficiency in chickens depending on the vitamin E content of the diet [26]. Assuming a reasonable intake of animal protein and grain products, most Nigerians should receive at least these levels in their dietary. However, three important qualifications should be considered before it is flatly assumed that persons in Nigeria need not worry about their selenium intake. First of all, there may be certain local pockets of low selenium soil which could contribute to a possible deficiency just as there are known high selenium areas that are hazardous for agricultural purposes [27].

Secondly, little is known regarding the availability and biopotency of selenium as it occurs naturally in foods. There is a wide variation in the ability of various selenium compounds to prevent liver necrosis in rats [28]. Morris and Levander noted that most active forms of selenium (such as sodium selenite) are also the least stable chemically, whereas the relatively inert elemental selenium is essentially without value in alleviating selenium deficiency diseases [29]. Therefore, the total selenium content of foods may not be a valid indicator of their nutritional value. Finally, Morris and Levander also noted that it must be recognized that many selenium compounds are quite volatile and could thus be lost as a result of food cooking or processing [29]. This suggests that selenium losses as a result of cooking or processing could be a major factor in determining the selenium content of an individual's diet.

Insufficient selenium intake may negatively affect the activity of several selenium-responsive enzymes and proteins Table 1. Even when severe, isolated selenium deficiency does not usually result in obvious clinical illness [30]. Yet, compared to subjects with adequate selenium status, selenium-deficient individuals might be more susceptible to additional physiological stresses [30]. Prolonged selenium deficiency may likely contribute to Keshan and Kashin-Beck diseases [31].

Conclusion

The present results suggest that the levels of selenium in north-central Nigerian staple foodstuffs are sufficient for good nutrition. Assuming a reasonable intake of animal protein and grain products, most of the populations in the research area should receive at least these levels in their diet. There is an obvious need for more research concerning the chemical forms of selenium that occur in foods, the biological effectiveness of these various forms, and the possible effects of cooking and processing on these forms.

Authors' contributions

KHJ and ZSCO developed the study design; ZSCO supervised the field work; KHJ and BJS conducted the biochemical analysis; KHJ analyzed and interpreted the data. All authors contributed to manuscript preparation. All authors read and approved the final manuscript.

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