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## Effect of soaking, boiling and frying on selenium content of major african fresh foods

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### Abstract

Selenium is an essential trace element that is crucial for normal functioning of human body systems and it is obtained from diet or dietary supplements. The concentration of selenium (Se) varies from soil-to-soil and therefore in various food types. We profiled the selenium concentration in the major African fresh foods and studied the effect of boiling, frying and overnight soaking on their selenium content. The foods were taken to the chemistry laboratory, processed for determining selenium concentration in the fresh foods and then subjected to the various treatments before determining their Se concentration using an atomic absorption spectrometer. Among the fresh foods studied, pumpkin seeds were found to have the highest Se concentration ( $109.25 \pm 0.125$  parts per billion (ppb)), while Irish potatoes had the lowest ( $16.25 \pm 0.125$  ppb). In most fresh foods studied, boiling and frying had the effect of reducing the Se concentration, except in yams that showed an increased concentration after frying. Overnight soaking of matooke (plantain bananas) and beans showed an effect of causing a considerable reduction in the Se concentration from  $36.375 \pm 0.281$  ppb and  $59.125 \pm 0.031$  ppb to  $14.03 \pm 0.441$  ppb and  $24.375 \pm 0.281$  ppb, respectively. Boiling and frying cause a significant reduction in Se concentration of most African fresh foods. Overnight soaking of matooke and beans caused further reduction in the selenium concentration of the fresh foods. This implies that people who pre-soak their food before cooking it are likely to lose more selenium than those who do not soak and may be more likely to suffer from Se deficiencies.

### Keywords

Selenium; African fresh-foods; Boiling; Frying; Overnight-soaking

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Conflict of Interest

The authors declare that there is no conflict of interest

## 1. Introduction

Selenium is known to be an essential trace element in the diets and for the normal body functions of every individual. It is solely obtained from our daily diet and inadequate intake or selenium deficiency can result in various disturbances in normal body function (1, 2).

Selenium exists in form of selenoproteins that are involved in a number of roles including acting as antioxidants, needed in normal DNA synthesis and for normal thyroid glandular functions (3). Selenium deficiency has been reported in individuals that suffer from endemic thyroid goiters in Uganda (4).

In addition, dietary supplementation with selenium is believed to improve the immune function of patients with chronic infections such as HIV (5) and those with chronic myopathies (6).

Attaining normal levels of selenium in the body is largely dependent on its concentration in the foods eaten as well as on its bioavailability (3). The selenium content in the foods consumed is in part dependent on its concentration in the soils where the food crops were grown (7).

Different foods will therefore, have varying selenium content due to the variation in selenium concentration of soils from different places or geographical parts of the country (8). On the other hand, post-harvest handling of food and its preparation before consumption may also affect the selenium content of the meals served in homes, restaurants and hotels. Failure to have adequate dietary intake of foods with sufficient content of selenium results into selenium deficiency for human beings that is characterized by experiencing disorders in muscular activity, abnormal thyroid functions, immune incompetence and reproductive malfunctions that may culminate into infertility in both men and women (9).

Dietary education and awareness regarding the importance of selenium as a micronutrient is therefore, necessary in order to prevent the disorders resulting from its deficiency (10). However, indiscriminate use of selenium dietary supplements should be avoided because of the potential to cause selenium toxicity that may result into complications such as hepatopathies (8).

It is therefore of critical importance to have the right balance of selenium within the body. This can be achieved by understanding the right methods to preserve sufficient levels of this micronutrient in commonly served meals. In this study, we aimed at profiling the selenium content in various fresh food sold in markets around Kampala, Uganda as well as highlighting the most optimum food preparation method by considering the effect of boiling, frying or applying direct heat and soaking of food, on its selenium concentration.

## 2. Materials and Methods

This was a laboratory-based experimental study. The fresh foods were conveniently selected from a lorry that supplies food to the three major food markets in Kampala including Kalerwe, Kasubi and Nakasero markets. Samples were collected and taken for further

processing and preparation in the nutrition unit of the physiology lab at Makerere University College of Health Sciences. Selected food preparations were then analysed in the chemistry lab for Selenium concentrations using Atomic Absorption Spectrometry combined with a Hydride Generation System for Selenium recovery. The study was approved by Makerere University School of Biomedical Sciences-Research and Ethics Committee (File No. SBS-861), on behalf of National Council of Science and Technology.

## 2.1. Sample Collection

Fresh samples of bananas/matooke (*Musa acuminata*), cassava (*manihot esculenta*), pumpkin (*Cucurbita moschata*), sweet potato (*Ipomoea batatas*), irish potato (*Solanum tuberosum*), yams (*Dioscoria spp.*) and beans (*Phaseolus vulgaris*) were conveniently procured from the lorries at farmers' market. The foods were cleaned to remove any soil residues using clean water. All foods except the beans were peeled properly leaving only the edible parts. They were then washed with deionised water to remove any potential contaminants. The beans were also washed with de-ionised water to remove any contaminants. They were then prepared using the most common methods for the several foods by either overnight soaking, boiling or frying in oil. The choice of the above foods was driven by the most commonly grown staple foods in the region.

## 2.2. Sample storage

Collected samples were protected from contamination or selenium loss during analysis. After cooling, 25 ml of the liquid samples were collected in sterile Falcon conical centrifuge tubes and kept at  $-20^{\circ}\text{C}$  until analysis. Solid samples were stored in sterile laboratory sample bags at room temperature ( $26^{\circ}\text{C}$ ) until preparation for analysis.

Sample preparation: Only the edible parts of the foods were prepared for analysis. 500 g of edible part of the foods were prepared either by overnight soaking in 500 ml of water or boiling/frying in cooking oil for at least 45 min until when ready. Fifteen (15) ml of the water in which the samples were soaked or boiled was stored in sterile falcon containers and stored for analysis. Five hundred grams of the prepared samples were heated in an oven until dry.

## 2.2. Sample preparation and analysis

The solid samples were homogenized to fine grain by a grinder while liquid samples were analysed directly.

Analyzing for selenium: All samples were prepared for analysis using a modified wet ashing procedure (11). Briefly, the analysis was done in two experiments on duplicates of samples. For all solid samples, 1.250 g of dried sample (dried in the oven for 24 h at  $103^{\circ}\text{C}$ ) was weighed and transferred to the destruction tube; 25 ml 65%  $\text{HNO}_3$  was then added with three boiling chips and a funnel placed on top of the destruction tube. The tube was then heated to  $100^{\circ}\text{C}$  and maintained for 1 h, heat was then increased to  $125^{\circ}\text{C}$ ,  $150^{\circ}\text{C}$ ,  $175^{\circ}\text{C}$  and  $200^{\circ}\text{C}$  maintaining the temperatures for 15 h at each change. The volume that was remaining was concentrated to 5 ml and left to cool. After cooling, 1 ml of 30%  $\text{H}_2\text{O}_2$  was added and destructed for 10 min. This destruction was repeated once. Then, after cooling

again, 3 ml of 30% H<sub>2</sub>O<sub>2</sub> was added and destructed for 10 min. Twenty-five (25 ml) of deionized water was then added, mixed and heated till boiling. The solution was cooled and transferred to a 250 ml volumetric flask, filled up to the mark, mixed and left to settle for 15 h. The absorbance of the supernatant was then measured by Atomic Absorption Spectrophotometry (AAS) using the Agilent 240 AA spectrophotometer series (Agilent Technologies, Santa Clara, California, USA) following standard procedures.

### 2.3. Statistical analysis:

All the data collected were cleaned and summarized in Excel spread sheets and analyzed for variations between individual food-selenium content by performing an unpaired t-test and plotting the relevant graph using GraphPad prism (version 8) software. For statistical significance of the difference in selenium content of the various fresh foods, a p-value of 0.05 was considered.

## 3. Results

The selenium concentration of pumpkin seeds was found to be the highest ( $109.25 \pm 0.125$  ppb), while Irish potatoes had the lowest concentration ( $16 \pm 0.125$  ppb) as shown in table 1 and Fig. 1, with significant difference between the highest and lowest, indicated by a p-value of  $p < 0.01$ , after comparing the selenium content of those harvested from the same garden. On boiling and frying for a duration of 60 min, there were significant reductions in the selenium content of most foods (with p-values of  $< 0.05$  as indicated in table 2 after boiling and frying, except in the case of yams and pumpkin pulp as shown in Fig. 2. The yams showed a significant increase in selenium concentration from  $18.75 \pm 0.13$  ppb to  $30.25 \pm 0.1$  (p-value of 0.005) after being fried, whereas the pumpkin pulp had an increase in selenium concentration from  $25.77 \pm 0.11$  ppb to  $31.5 \pm 0.5$  ppb (p-value of 0.0092) following boiling (Fig. 2 and table 2). On comparing the effect of boiling and frying the selenium content of beans and Irish potatoes.

We found a surprisingly significant increase in the selenium content of beans from  $59.13 \pm 0.031$  to  $87.88$  ppb and  $82.5 \pm 0.5$  ppb (p-values of potatoes only showed an increase in selenium from  $16.25 \pm 0.13$  ppb to  $26.38 \pm 0.031$  ppb following boiling, but not on frying (shown in Fig. 3), and the water used for boiling the beans also had a significantly increased selenium concentration of  $2.66 \pm 0.0032$  µg/l compared to that for matooke (of  $0.33 \pm 0$  µg/l) and Irish potatoes (of  $0.35 \pm 0$  µg/l) (p-value of 0.0003) as indicated in Fig. 5 and table 2.

On overnight soaking of matooke (plantain bananas) and beans as practiced by some people either under medical instructions or as a means of softening the beans before boiling them, there was a reduction in the selenium concentration of both matooke (from  $36.375 \pm 0.281$  ppb to  $14.03 \pm 0.442$  ppb) and beans (from  $59.13 \pm 0.0313$  ppb to  $24.375 \pm 0.281$  ppb), which was statistically significant, at p-values of 0.0049 for beans and 0.0405 for matooke (Fig. 4).

The concentration of selenium in the water used both in boiling and soaking of the various foods was also determined as indicated by Fig. 5. Apart from water used to boil or soak

beans, there was only slight increase in the concentration of water used to boil or soak the other foods, as indicated in Fig. 5.

#### 4. Discussion

Our results indicate that among all the fresh foods sampled, the pumpkin seeds have the highest selenium concentration of about 109.25 ppb or 4.2 µg/g compared to all the rest of the foods.

This is still a relatively much lower concentration of selenium compared to that found in some other plants, which have been for a long time known as 'selenium accumulators' with more than 25 µg/g and these include plants like *aster*, *triplex*, *Brassica juncea* (Indian Mustard) and *Stanleya pinnata* (12,13). There was a general reduction in the selenium concentration of most fresh foods following boiling and frying. We purposely chose these two methods in order to reflect the most common ways of food preparation in most African countries and see if they have any impact in terms of changing the concentration of this essential micronutrient-selenium in people's diets. This reduction in the selenium content of these foods following boiling and frying, could imply that selenium is leached or escapes into the water used in boiling or the cooking-oil used in frying of the food. Indeed, we detected a slight increase in the selenium-concentration of the water used to boil the various foods. This result is in contradiction to a previous report that suggested that general cooking methods do not cause much change in selenium concentrations of most foods following food preparation methods such as cooking and frying (14). Moreover, it has also been reported previously that cooking and frying have the effect of reducing selenium content of foods that have substantially higher levels of the micronutrient (15–17).

Frying has a much smaller effect of reducing the selenium concentration than cooking, as more selenium is leached into the water used in cooking than what is leached into the cooking oil during frying (15–17). However, we could not determine the selenium concentration in the cooking oil used to fry the foods, as our equipment was unable to read if there were any selenium levels leached there.

Overnight soaking of matooke and beans, only caused a considerable reduction in selenium content of the food. This practice of presoaking food before cooking it is commonly practiced by people who want to soften foods like beans in order to shorten the cooking time, as well as by patients of chronic kidney disease that are advised to presoak matooke in order to decrease the concentration of potassium before cooking it for their meals (18). As such, we also expected to see a reduction in the selenium concentration following overnight soaking of matooke and beans, but we were surprised to see quite significant reductions in both foods from  $36.375 \pm 0.28125$  ppb and  $59.125 \pm 0.03125$  ppb to  $14.03 \pm 0.4418$  ppb and  $24.375 \pm 0.28125$  ppb, respectively (p-value of 0.0049 for beans and 0.0405 for matooke). This is because soaking alone has been previously reported not to be an effective method of releasing metal ions from food without the complementally effect of boiling the food (18). This could be explained by the possibility that there might be easier escape or leaching of selenium into the water compared to other ions such as potassium from fresh foods.

Indeed, we did observe a slight increase in selenium concentration of the water used for soaking these two foods, with significantly higher increase in the water used to boil or soak beans as compared to that used for matooke. This could suggest that there is additional release or leaching of selenium from the coat of the beans, unlike the case for Matooke which were peeled before boiling or soaking.

#### 4.1. Limitations to this study

The major limitations include the fact that the foods were boiled in ordinary tap water instead of using deionized water, which is not contaminated with any ions such as those of selenium. This was done in a way of trying to mimic the conditions under which food is prepared by most Africans, in which case, no one ever cooks their meals using deionized water. However, the concentration of selenium in the plane/unused water was determined prior to experimentation and was adjusted for in the final analysis, when determining the amount of selenium released into the water used in the boiling or soaking of the respective foods. In addition, we were also unable to determine the amount of selenium released into the cooking oil that was used to fry the foods, as our machine was not able to detect selenium in the oily medium. In addition, since Selenium can exist both in organic form (such as gamma-glutamyl methylselenocysteine, Selenocystein & Selenomethionine) and in inorganic form (such as selenate or selenite) (3), it would have been prudent to verify if all the organic forms of selenium in the tested foods were converted into the inorganic form that is easily quantified by the atomic absorption spectrometer. Unfortunately, we were unable to confirm this important aspect during our experimental procedures. So, it could be possible that some of the variations in selenium concentrations of foods following boiling and frying, as seen in yams might be due to increased conversion or release of selenium from the organic form to inorganic form in particular foods. Therefore, further studies are recommended in order to confirm the possibility of increased conversion of organic forms of selenium into the inorganic form following boiling or frying of these foods.

## 5. Conclusion

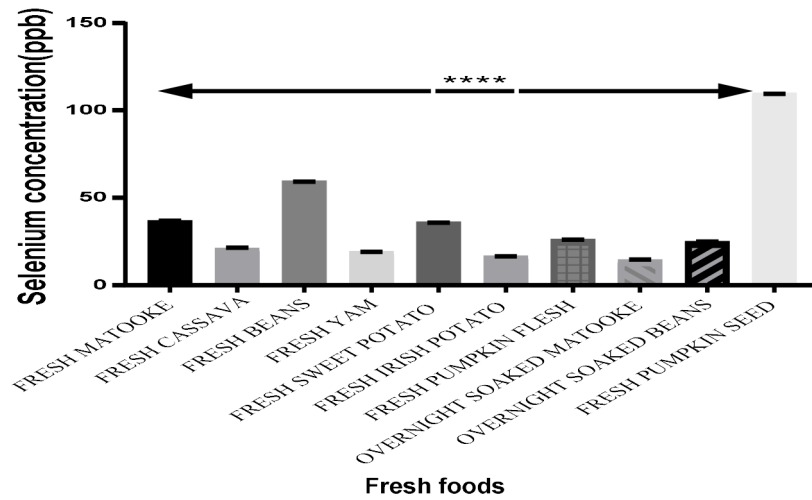
Boiling and frying cause a significant reduction in selenium concentration of most fresh foods sold in food markets around Kampala. In addition, overnight soaking of matooke and beans caused further reduction in the selenium concentration of the two fresh foods. This implies that the people who pre-soak their foods (such as beans and matooke) before cooking are likely to lose more selenium during food preparation than those who do not pre-soak food.

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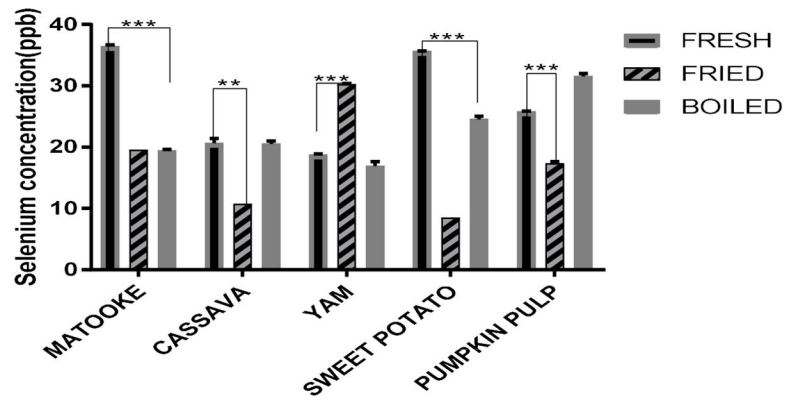
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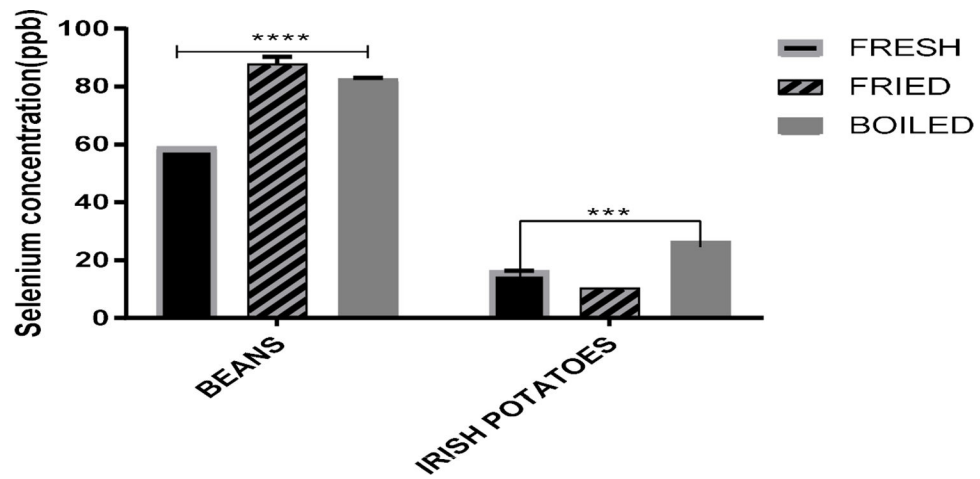
**Figure 1.** Selenium concentration in the major African fresh-foods. The concentration is reported in parts per billion (ppb). \*\*\*\* means p-value of less than 0.008 for the comparison between the highest and lowest selenium concentration across all foods. The concentration of selenium is represented as means  $\pm$ SEM from two experiments.



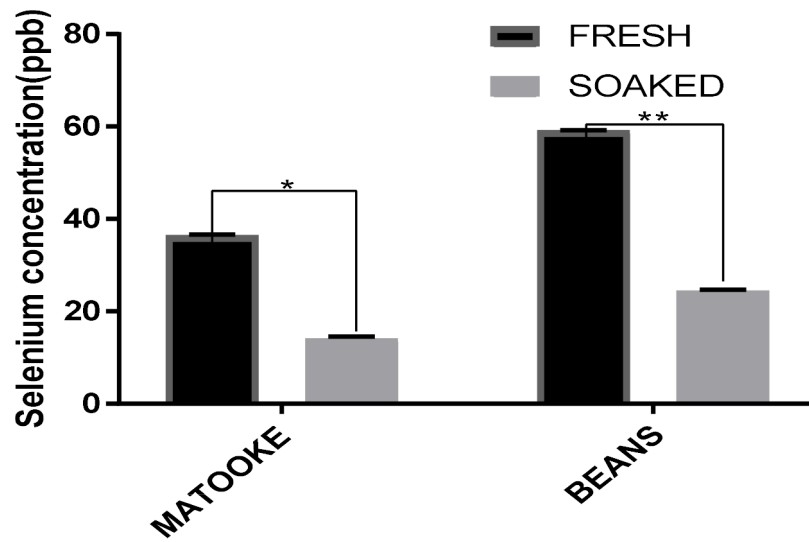


**Figure 2.**

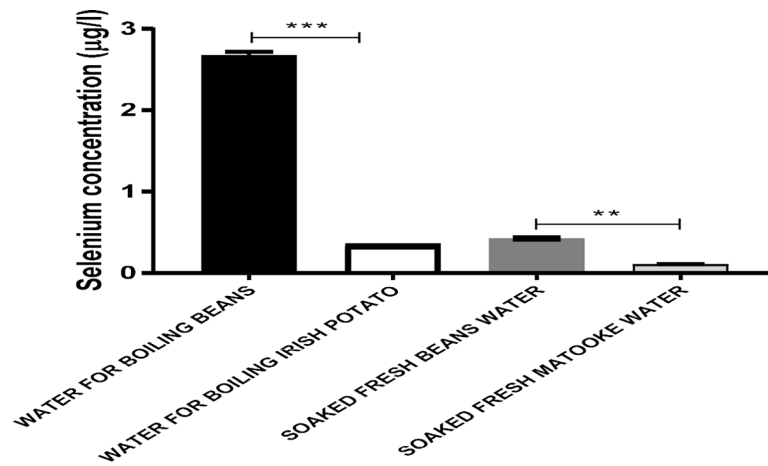
The effect of boiling and frying the fresh foods on their Selenium concentration. The Se concentration is reported in parts per billion (ppb). \*\*\* means p-value of less than 0.0009; \*\* refers to a p-value of 0.009. The concentration of selenium is represented as means  $\pm$ SEM from two experiments.



**Figure 3.** Comparison between the effect of boiling and frying on the Selenium content of beans and Irish potatoes. The Selenium concentration is reported in parts per billion (ppb). \*\*\*\* means p-value of less than 0.0001; \*\*\* refers to a p-value of 0.005. The concentration of selenium is represented as means  $\pm$ SEM from two experiments.



**Figure 4.** Comparison between the effect of overnight soaking of Matooke (plantain bananas) and beans. The Selenium concentration is reported in parts per billion (ppb). \* = Significant P-value of 0.0405; \*\* = very significant p-value of 0.0049. The concentration of selenium is represented as means  $\pm$ SEM from two experiments.



**Figure 5.** Selenium concentration in the water used for boiling or soaking the various foods. The Selenium concentration is reported in micrograms per liter ( $\mu\text{g/l}$ ). \*\* = Significant P-value of 0.0013; \*\*\* = very significant p-value of 0.0003. The concentration of selenium is represented as means  $\pm$ SEM from two experiments.

Selenium concentration in various fresh foods and the same foods after frying or boiling, reported as micrograms per gram weight of food ( $\mu\text{g/g}$ ) and parts per billion (ppb)

**Table 1.**

Food type	Se Mean conc. ( $\mu\text{g/g}$ )	$\pm\text{SEM}$	Se mean conc. (ppb)	$\pm\text{SEM}$
Fresh matooke	1.315	0.048	36.375	0.28125
Fresh cassava	0.825	0.00125	20.625	0.78125
Fresh yam	0.73	0.0018	18.75	0.125
Fresh sweet potato	1.415	0.00045	35.625	0.03125
Fresh pumpkin flesh	0.925	0.0265	25.765	0.11045
Fresh pumpkin seed	4.19	0.0722	109.25	0.125
Overnight soaked matooke	0.53	0.005	14.03	0.4418
Overnight soaked beans	0.895	0.018	24.375	0.28125
Fresh beans	2.335	0.0025	59.125	0.03125
Fresh Irish potato	0.63	0.0018	16.25	0.125
<b>Fried</b>				
Fried matooke	0.79	0.0002	19.55	0.005
Fried cassava	0.44	0.0002	10.775	0.00125
Fried Yam	1.35	0.045	30.25	0.125
Fried sweet potato	0.34	0	8.5	0
Fried pumpkin flesh/pulp	0.705	0.00005	17.375	0.28125
Fried beans	3.52	0.004	87.875	2.53125
Fried Irish potato	0.705	0.00005	10.55	0.005
<b>Boiled</b>				
Boiled matooke	0.765	0.00125	19.375	0.28125
Boiled cassava	0.885	0.00045	20.5	0.5
Boiled yam	0.675	0.00125	16.875	0.78125
Boiled sweet potato	0.93	0.0018	24.5	0.5
Boiled pumpkin flesh/pulp	1.34	0.018	31.5	0.5
Boiled beans	3.29	0.0002	82.5	0.5

Food type	Se Mean conc. (µg/g)	±SEM	Se mean conc. (ppb)	± SEM
Boiled Irish potato	1.03	0.0013	26.375	0.03125

Definitions: ppb = parts per billion; µg/g = microgram per gram weight of food; SEM = Standard Error of Mean

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**Table 2.** Comparison between selenium concentrations in parts per billion (ppb) of fresh and boiled or fried foods

Food type	Se Mean conc. (ppb)	±SEM	Se mean conc. (ppb) in boiled foods	± SEM	p- value	Se mean conc. (ppb) in fried foods	± SEM	p-value
FRESH MATOKE	36.375	0.28125	19.375	0.28125	** 0.001	19.55	0.005	*** 0.0005
FRESH CASSAVA	20.625	0.78125	20.5	0.5	NS 0.8902	10.775	0.00125	** 0.004
FRESH YAM	18.75	0.125	16.875	0.78125	NS 0.1083	30.25	0.125	*** 0.0009
FRESH SWEET POTATO	35.625	0.03125	24.5	0.5	** 0.0021	8.5	0	*** 0.0001
FRESH PUMPKIN FLESH/PULP	25.765	0.11045	31.5	0.5	** 0.0092	17.375	0.28125	** 0.0028
FRESH PUMPKIN SEED	109.25	0.125	-	-	-	-	-	-
OVERNIGHT SOAKED MATOKE	14.03	0.4418	19.375	0.28125	-	19.55	0.005	-
OVERNIGHT SOAKED BEANS	24.375	0.28125	82.5	0.5	-	87.875	2.53125	-
FRESH BEANS	59.125	0.03125	82.5	0.5	*** 0.0001	87.875	2.53125	*** 0.0001
FRESH IRISH POTATO	16.25	0.125	26.375	0.03125	*** 0.0003	10.55	0.005	*** 0.0001

Definitions: Conc. = concentration; ppb = parts per billion; SEM= Standard Error of Mean