Working together to eliminate cyanide poisoning, konzo, tropical ataxic neuropathy (TAN) and neurolathyrism

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Beyond the Millenium Development Goals: new agenda

Health for All by 2000 from the Alma Ata Declaration of 1978 was an unachieved goal. Then, from 2000 to 2015, many milestones were covered but unlikely to achieve the eight Millennium Development Goals (MDGs):

- MDG 1: Eradicate Extreme Hunger
- MDG 2: Achieve Universal Primary Education
- MDG 3: Promote Gender Equality and Empower Women
- MDG 4: Reduce Child Mortality
- MDG 5: Improve Maternal Health
- MDG 6: Combat HIV AIDS Malaria and Other Diseases
- MDG 7: Ensure Environmental Sustainability
- MDG 8: Develop a Global Partnership for Development

The eight MDGs included five nutrition and health-related goals (1,4,5,6,7) to be linked to konzo and neurolathyrism prevention.

The latest UN reports on MDGs suggested that only some countries did in reality live up to the goals but other countries, especially in Sub-Saharan Africa missed the goals despite making progress.

Since 2015, a set of Sustainable Development Goals (SDG) to achieve by the year 2030, as the successor framework to the MDGs, has been developed by the international community with17 goals and 169 targets. The new agenda, which builds on the MDG, aims to be relevant to all countries and focuses on improving equity to meet the needs of women, children and the poorest, most disadvantaged people.

- SDG 1 End poverty in all its forms everywhere

- SDG 2 End hunger, achieve food security and improved nutrition and promote sustainable agriculture
- SDG 3 Ensure healthy lives and promote well-being for all at all ages
- SDG 4 Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all
- SDG 5 Achieve gender equality and empower all women and girls
- SDG 6 Ensure availability and sustainable management of water and sanitation for all
- SDG 7 Ensure access to affordable, reliable, sustainable and modern energy for all
- SDG 8 Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all
- SDG 9 Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation
- SDG 10 Reduce inequality within and among countries
- SDG 11 Make cities and human settlements inclusive, safe, resilient and sustainable
- SDG 12 Ensure sustainable consumption and production patterns
- SDG 13 Take urgent action to combat climate change and its impacts
- SDG 14 Conserve and sustainably use the oceans, seas and marine resources for sustainable development
- SDG 15 Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land desertification and halt biodiversity loss
- SDG 16 Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions for all
- SDG 17 Strengthen the means of implementation and revitalize the global partnership for sustainable development

Nutrition and Health have a central place in SDG; almost all of the SDG are directly related to nutrition and health or will contribute to nutrition and health indirectly.

At least 12 of the 17 Sustainable Development Goals contain indicators that are highly relevant for nutrition, reflecting nutrition's central role in sustainable development. Improved nutrition is the platform for progress in health (including nutrition related disorders such konzo and neurolathyrism), education, employment, female empowerment, and poverty and inequality reduction. In turn, poverty and inequality, water, sanitation and hygiene, education, food systems, climate change, social protection, and agriculture all have an important impact on nutrition outcomes.

The global nutrition report 2016 (IIFPRI, 2016) shows that women's power and status constitute a particularly important driver of malnutrition: mothers age 18 or under are more likely to have stunted children, and children are less likely to be stunted if their mother has secondary education. It is thus important to incorporate nutrition targets into development, education and social sectors, where many governments spend more than 30 percent of their budgets, and to measure the impacts of spending in these sectors on people's nutrition.

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New mild method to produce pounded cassava leaves free of cyanogens

Cassava leaves are used widely in Africa as a vegetable. The Congolese people of Central Africa are the greatest users with more than 60% of all vegetables consumed being cassava leaves. Elsewhere cassava leaves are a significant part of the diet in adjacent countries including East Africa and Madagascar (40- 60%) and below 40% consumption in West Africa, with the exception of Liberia, Sierra Leone and Guinea where there is high consumption.[1] These authors state that cassava leaves should be given as much importance as the roots. The Congolese consider that cassava is "all sufficient" because they get "bread (carbohydrate) from the roots and meat (protein) from the leaves."[1] Cassava leaves are a very good source of protein, vitamins and minerals[2,3] However the protein is deficient in the essential S-containing amino acids methionine and cysteine/cystine, which

are needed for detoxification of cyanide (CN) which is present in cassava leaves to thiocyanate (SCN) in the body[4].

Cassava leaves contain large amounts (100-2000 ppm) of the cyanogenic glucosides, linamarin and a small amount of lotaustralin,[3,5] that must be removed before consumption, to avoid cyanide poisoning. Traditional methods are largely based on washing the leaves followed by pounding in a pestle and mortar and boiling in water for long periods of 1-2 h. The long boiling treatment causes loss of protein[2-4] and very great loss of vitamins.[2]

Our first attempt to develop a mild method of removal of cyanogens to conserve vitamins and protein involved the traditional pounding followed by two water washes at room temperature that reduced the total cyanide content to 3% of its initial value.[6] The second attempt involved pounding of leaves in a pestle and mortar followed by standing for 2 h at 50°C, which allowed the enzyme linamarase to catalyse hydrolysis of linamarin to acetone cyanohydrin, that was hydrolysed to hydrogen cyanide and acetone catalysed by the enzyme hydroxynitrile lyase present in the leaves.[7] This additional step is analogous to the key step in the wetting method used to remove cyanogens from cassava flour,[8-10] and is the critical step in our improved method. Subsequent washing removed virtually all cyanogens and the pounded cassava leaves retained their bright green colour.[7] However they had an unpleasant taste and odour.

The third study made at PRONANUT involved the adaptation of the method developed in the laboratory in Australia to suit African conditions and tastes. Two varieties of cassava leaves (bitter and "false") were obtained from the markets in Kinshasa, DRC. False cassava is a variety of cassava that produces no tubers, but is grown for its leaves. The leaves were washed, pounded in the traditional way and five samples were taken for duplicate analyses for total cyanide using cyanide kit B2.[11,12] The pounded leaves were placed on flat plates in the sun for 2 h and then boiled in water for 5, 10 or 15 min and total cyanide analyses made as before. The mean total cyanide content after pounding was 88 ppm and after pounding, standing for 2 h in the sun and boiling for 5, 10 or 15 min was 8, 6 and 5 ppm respectively. The maximum WHO safe level

for cassava flour is 10 ppm.[13] Nine women from PRONANUT assessed the two pounded cassava leaf samples of bitter and "false" cassava. They found that 5 min boiling was insufficient to remove the unpleasant fresh odour and taste of pounded cassava. After boiling for 10 min the majority of assessors found that the taste was good and the odour was removed and after 15 min boiling all agreed that the odour was removed, the taste was good and the pounded leaves were acceptable for consumption, particularly with addition of tomatoes, onions etc to improve the flavour of the pounded leaves. The green colour remained after 5 min boiling, was still partly there after 10 min boiling, but was not present after 15 min boiling.

Umuhozariho et al[2] found that three different samples of pounded cassava leaves after 15 min boiling had an average 27% reduction in ascorbic acid, 31% reduction in beta-carotene and no reduction in crude protein. After 30 min boiling the reduction in ascorbic acid, beta-carotene and crude protein was 98%, 34% and 6% respectively,[2] compared with Diasolua Ngudi et al[4] who found a protein and methionine loss of 58% and 71% respectively. It is therefore clear that boiling for 10-15 min using our adapted mild method would reduce the ascorbic acid and beta-carotene content by about 30%, not affect the crude protein content and reduce the total cyanide content to a safe level of about 6 ppm.

The new mild method adapted to African conditions is as follows:

Cassava leaves are washed in water, pounded in a pestle and mortar in the traditional way and the pounded leaves are placed in a dish in the sun for 2 h. The green pounded leaves are boiled in water for 10-15 min (no longer). They are ready to eat.

The method has removed virtually all cyanogens, reduced the content of vitamin C (ascorbic acid) and vitamin A (beta-carotene is a precursor of vitamin A) by about 30% and produced pounded cassava leaves that are of superior quality to the pounded leaves produced by traditional methods.[1,3] The new method takes about the same time as the traditional method and the reduced cooking time from 1-2 h to 10-15 min saves on fuel costs. We hope that the new mild method adapted to African conditions will be used widely to improve the nutrition of the people and in this way help to prevent diseases due

to malnutrition and high cyanide intake such as konzo.[14]

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Is stress a factor in the susceptibility to neurolathyrism?

Imposing stress in rat pups exposed to β-ODAP resulted in a much higher incidence of neurolathyritic symptoms. Transient hemorrhage in the lower spinal cord was observed in affected animals. Reviewing historical reports of epidemics of neurolathyrism supports the hypothesis that stress could be a risk factor for the susceptibility to neurolathyrism. Oxidative stress was also identified as a plausible risk factor, together with poverty, illiteracy and physical labour.

The epidemiology of neurolathyrism has been studied in India, Bangladesh and Ethiopia. Poverty, illiteracy, physical labour, young age and male gender were invariably identified as risk factors, while overconsumption of grass pea seed was seen as the obvious cause. Fermentation of grass pea as in the traditional Ethiopian injera can reduce the risk for neurolathyrism by breakdown of β-ODAP and improves the balance of essential amino acids. Consumption of certain foods richer in sulfur amino acids or condiments with antioxidant capacity together with grass pea can also have effects on the susceptibility to neurolathyrism.[1] The variability in post-harvest treatment, in food preparations and the composition of the total diet may explain why a direct ratio between intake of grass pea or β-ODAP and susceptibility to neurolathyrism, or a threshold level was not established. Even during epidemics of neurolathyrism, at least 94% of the population remains unaffected. The well documented exception is the prisoners of war camp at Vapniarca, where 60 % of the inmates were affected. Those inmates were subjected to forced physical labour and the stressful risk of imminent death at the hands of their jailors.

Partly because of individual variability and the low susceptibility, a practical animal model for neurolathyrism is difficult to develop. The often used day-old chicks develop neurological symptoms after intra-peritoneal injection of β-ODAP, but these symptoms are different from human neurolathyrism and are reversible as opposed to the irreversible symptoms of neurolathyrism. When new-born rat pups were subjected to subcutaneous injections with 400mg/kg β-ODAP (300 mg/kg β-ODAP itself), various neurological symptoms developed and β-ODAP could be found in the spinal cord.[2] At this early age, the blood/brain barrier protecting

the central nervous system from reactive chemicals in the blood stream is not fully mature and β-ODAP can have leaked into the spinal cord. The highest concentration of β-ODAP was found in the lower part of the spinal cord, where the blood/brain barrier matures slower. When the injections are repeated for six consecutive days, irreversible paraparesis of the hind legs occurred in 3% of the young animals.[2]

When the rat pups received the same treatment with subcutaneous injections of ~300mg/kg β-ODAP and in addition were put under stress, a 4.6- fold higher percentage of rat pups developed paraparesis of the hind legs after 3 to 5 daily injections. Histological examination demonstrated greater changes in the lumbosacral part of the spinal cord. Transient hemorrhage in this lower part of the spinal cord clearly indicates a malfunction of the blood/brain barrier. This is a highly selective permeability **barrier** that separates the circulating **blood** from the **brain** extracellular fluid in the central nervous system (CNS) and prevents the entry of toxic molecules into the CNS. All rat pups developing paraparesis of the legs showed this hemorrhage in the lower spinal cord, but none of the controls.[3] The vascular endothelial growth factor (VEGF) system normally ensures the integrity of the blood/brain barrier. Interestingly, while the level of VEGF was normal in paraparetic rats and controls, the level of VEGF-receptors was much reduced in lumbosacral spinal cord of the paraparetic rats.[3] Loss of integrity of the blood/brain barrier in the lower part of the spinal cord may permit the entry of β-ODAP into that part of the CNS. The fact that the hemorrhage is transient suggests that there is a discrete period of dysfunction of the blood/brain barrier that coincides with the onset of the clinical symptoms. This observation may explain the sudden onset of neurolathyrism and its non-progressive nature.

In an experimental cellular model system of the blood/brain barrier, using brain capillary endothelial cells in non-contact co-culture with glial cells, acute exposure to 100 μM – 5 mM β-ODAP during 2 h had no effect. Chronic treatment with 200 μM β-ODAP during 6 days resulted in significantly increased permeability, while 200 μM of L-glutamate had no effect.[4]

Blood/brain barrier dysfunction receives increasing scientific attention. It is linked to several neurological disorders including neurodegeneration, with oxidative stress often mentioned as cause.[5] Also in neurolathyrism, oxidative stress has been mentioned as a result of deficiency in the diet of sulfur amino acids leading to decrease in glutathione. When considering the socio-economic conditions prevailing during epidemics of neurolathyrism, it seems logic that those populations were under stress, especially during periods of food insufficiency. The extreme stress of inmates in a forced labor camp during war-time may explain the extremely high incidence of neurolathyrism in the Vapniarca camp. Chronic intake of β-ODAP may also contribute to a transient dysfunction of the blood/brain barrier that is evidenced by transient hemorrhage in the lower spinal cord of experimental rat pups. It is intriguing that β-ODAP is also present in Ginseng roots where it is named dencichine. The property of dencichine (β-ODAP) as being hemostatic may have to be taken into account in the final picture of onset of neurolathyrism. Further scientific evidence may be needed to confirm this hypothesis of stress as an important risk factor in neurolathyrism incidence.

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Evaluation of common processing methods on selected minerals, anti-nutrient and its bioavailability of seven cultivars of cassava tubers (*Manihot esculenta C.*) grown in Ethiopia

Abstract

We investigated the effect of common processing methods on selected antinutrients, minerals and its predicted bioavailability of seven cassava cultivars collected at the same stage of maturity from different areas of Ethiopia. The root flours were analyzed for iron, zinc, calcium, phytate and oxalate. The raw, boiled, sun-dried and fermented flours of the seven cultivars contained 1.47, 1.00, 1.01 and 0.99 mg/100 g zinc; 259.08, 143.44, 109.39 and 114.93 mg/100 g calcium and 1.44, 1.20, 1.22 and 1.36 mg/100 g iron; 27.16, 17.75, 18.13 and 10.28 mg/100 g oxalate; 803.95, 655.15, 403.80 and 425.80 mg/100 g phytate respectively. The three processing techniques are found to be effective in reducing phytate and oxalate levels of all seven cultivars. The mean values of molar ratio were 0.22, 0.42,

0.22 and 0.23 for phytate to calcium; 0.12, 0.19, 0.16 and 0.10 for oxalate: calcium for raw, boiled, sun-dried and fermented cassava flours, respectively. All other values of phytate: calcium molar ratio are below the critical value (<0.5) except for two boiled cultivars of 5538-19 (1.36) and 44/72-NW (0.59). This indicates the availability of calcium in most of the flours.

Key words: mineral, processing methods, bioavailability, phytate, molar ratio, oxalate

Introduction

Cassava (Manihot esculenta C.) was introduced to Ethiopia at the middle of the nineteenth century.[1] Cassava is an essential part of the diet for more than half a billion people in the world and an important source of income for farmers in several African countries.[2,3] However, in Ethiopia it is considered as less important and consumption and processing of cassava in the country is in a rather primitive stage as compared to many African countries.

When raw cassava or inadequately processed cassava was consumed different health problems may develop, including the irreversibly crippling neurodegenerative disease konzo .[4] Cassava contains anti-nutrients like tannin, oxalate and phytate which inhibit the absorption of minerals .[5]

There is a lack of information on the area and production of cassava in Ethiopia, however, the crop has been in cultivation, particularly, in South, South West, and Western parts of the country since its introduction .[6] Methods are needed for enhancing the nutrient content and at the same time reducing the anti-nutrients without adversely affecting the acceptability of the crop. The relative cheapness of cassava roots has a selective effect by providing a cheap alternative for the low-income group of consumers. At this stage of our study we investigated the effect of common processing methods on iron, zinc, calcium and its bioavailability, and phytate and oxalate of cassava root cultivars grown in Ethiopia.

Materials and Methods

The seven cultivars of 12 to 14 months old cassava roots were taken from Western, Southern and Northern parts of the country. They were manually harvested, packed into a sack, and transported within one day to the laboratory. The samples were processed in the CFSN laboratory while the chemical analyses were done in the Ethiopian Public Health Institute (EPHI) laboratory, Addis Ababa, Ethiopia. Using published standard procedures, the tubers were processed by sun-drying, boiling and fermentation. After each processing, the material was dried to less than 12% moisture content. After dry ashing, Ca, Fe and Zn were determined using Flame Absorption Spectrophotometry. **Bioavailability of minerals** was estimated on the basis of molar ratios, that were calculated by dividing the mole of anti-nutritional factors to mole of minerals using the techniques of Morris and Ellis.[7]

Results and discussions

Effect of processing methods on minerals content in cassava flours

Zinc

The average zinc content for the seven processed cassava roots cultivars was 1.00, 1.01 and 0.99 mg/100 g for the boiled, sun-dried and fermented flours, respectively. This is high er than the value given in the Food Composition Table of Ethiopia for cassava (0.20 mg/100 g) .[8] The average sun-dried flours Zn contents were significantly (P<0.05) higher than other processed flours. Whereas, the zinc content of unprocessed cassava roots was measured in the marginal mean value of 1.47 mg/100 g fresh weight basis. **Calcium**

The average calcium contents of the processed cassava roots flour were 143.44, 109.39 and 114.93 mg/100 g for the boiled, sun-dried and fermented flours, respectively. The average Ca content of boiled flours was found to be the highest of all other methods. However, the calcium content of the seven cultivars was lower than the processed cassava leaves flour mean value (210 mg/100 g) reported by Fasuyi.[9]

The calcium content of unprocessed cassava roots was considerably higher (259.08 mg/100 g) than after processing.

Iron

The average iron contents of processed cassava root flours were 1.20, 1.22 and 1.36 mg/100 g that corresponds to boiled, sun-dried and fermented flours, respectively. The average Fe content of fermented flours were significantly (P<0.05) higher than boiled and sun-dried flours. The iron content of unprocessed cassava roots cultivars was significantly (P<0.05) higher than processed ones except for the fermented cultivars 28 and 192 having the same value.

Anti-nutritional factors levels.

Phytate

Phytic acid has a negative effect on the bioavailability of minerals and is considered an anti-nutritional factor, it is a common storage

form of phosphorus in plant seeds, tubers and fruits. The average phytate levels of processed cassava tuber flour was found to be 655.15, 403.80 and 425.80 mg/100 g, for the boiled, sun-dried and fermented flours, respectively. The average level after processing methods over levels in cultivars are significantly (P<0.05) different. The mean for processed sun-dried (403.80 mg/100 g) being the lowest followed by fermented and boiled. The phytate level was highest for boiled cultivar of Gamo (910.66 ± 0.40 mg/100 g) followed by Koree (818.70 ± 1.56 mg/100 g) and the lowest belongs to fermented cultivar 44/72-NW (108.57 ± 2.1 mg/100 g). The average level obtained from the various cultivars over levels of processing methods are significantly (P<0.05) different with the cultivar Hayik (269 mg/100 g) being the lowest. The average phytate content of processed cassava flour was significantly different (P<0.05) from one another except for the phytate content of boiled (44/72-NW) and sun-dried (Hayik). The complexing of phytate with nutritionally essential elements and the possibility of interference with proteolytic digestion have been suggested as responsible for the anti-nutritional activity. One of the factors is a negatively charged phosphate in the phytate molecule that binds minerals and inhibits absorption. The variability of phytate content in cassava tubers is not only due to cultivar factors but also it might be due to the total phosphorous content, found in soil and fertilizers, which can influence the phytic acid concentration.[10] The phytate intake of Ethiopian's food is still unknown, consequently the effect of phytate in local food of the country is not predictable. The phytate content of unprocessed cassava tubers (947.41 mg/100 g) was significantly (P<0.05) higher than in processed ones. Among the processing methods, sun-drying and fermentation are more effective to reduce the phytate levels when compared to boiling. The decrease in the phytate content of the fermented cassava flour for all the seven cultivars could possibly be attributed to the activity of the enzyme phytase. This enzyme is capable of hydrolyzing phytate, thereby decreasing the phytate content of the flour.[11,12] The decrease in phytate content during fermentation and boiling may be partly due to either the formation of insoluble complexes, such as phytate-protein and phytate-mineral.[13] Moreover, the Inositol hexaphosphate might also be hydrolyzed to penta- and tetra- phosphates and then leached out when boiled or fermented. The high content of phytate of nutritional significance is lowering the availability of many other essential dietary minerals. Thus, reduction of phytate is expected to enhance the bioavailability of dietary minerals.[14] On the other hand, there is evidence that dietary phytate at low level may have a beneficial role as antioxidant, anti-carcinogens and likely play an important role in controlling hypercholesterolemia and atherosclerosis.[15]

Oxalate

The ingestion of oxalic acid can cause the formation of calcium oxalate which is insoluble at physiological pH and can be deposited in the brain and kidney tubules. The lethal dose for oxalates in adults is estimated (143 - 428 mg/kg).[16] The average levels of oxalate in processed cassava tuber flour were 27.16, 17.75, 18.13 and 10.28 mg/100 g for the raw, boiled, sun-dried and fermented flours, respectively. The effects of cultivars and processing methods were found to be significant (P<0.05). Similarly, the interaction of cultivars and processing methods was significant (P<0.05). The highest reduction of oxalate is found for fermented, followed by boiled and sun-dried flours. This average oxalate level variation observed is much higher than the range reported in a previous study (1.35 - 2.88 mg/100 g) for leaves .[17] The lowest average oxalate level was recorded for cultivar 44/72-NW (3.18 ± 0.00 mg/100 g) on dry weight basis after fermentation followed by sun-dried cultivar 44/72-NW (3.35 ± 0.22 mg/100 g). The highest level was observed for boiled cultivar Koree (29.00 ± 0.00 mg/100 g) cassava tubers flour. The processed cultivars flour has shown significant (P<0.05) variability in cell means oxalate levels except for the cultivar Hayik (13.04 ± 0.06 mg/100 g). Whereas the highest and lowest average oxalate levels found were 30.63 ± 0.21 and 19.81 ± 0.26 mg/100 g flour in Koree and 192 unprocessed cassava cultivars, respectively.

--Bioavailability of Ca, Zn and Fe in the 7 cultivars of cassava tuber flours

To maintain the dietary mineral balance, it is not only the intake of a mineral that needs consideration, but more importantly the amount that is available to be absorbed. Thus, the bioavailability of minerals could be predicted by molar ratio of anti-nutritional factors interfering with bioavailability to mineral value.[18] Many metal ions form insoluble precipitates with oxalate, a prominent example being calcium oxalate, the primary constituent of the most common kind of kidney stones.

Molar ratio of oxalate: calcium (ox to Ca)

In Table 1 the average values for molar ratio of oxalate: calcium in cassava tuber cultivars are given: we found 0.12, 0.19, 0.16 and 0.10 for the raw tubers flour, the boiled, sun-dried and fermented flours, respectively. The interaction between cultivars and processing effects were significant (P<0.05). Similarly, the effect of cultivars and processing methods were significant (P<0.05). The average values of molar ratio of ox: Ca were found to increase in boiled and sun-dried flours when compared to raw. This might be due to calcium ions bound to and made insoluble by other anti-nutrient factors in the flours. The highest and lowest averages were found for boiled cultivar 44/72-NW (0.45 ± 0.00) and fermented cultivars 5538-19 and 44/72-NW which had similar value (0.03 ± 0.00), respectively. The present study result of the seven processed and unprocessed cassava tuber cultivars shows lower molar ratio of oxalate to calcium which is less than the critical value (<2.5) reported by Hassan, et al..[19] Therefore, the oxalate levels found in these cultivars of cassava roots do not endanger the uptake of the calcium contained in it. In general, the reduced oxalate content by processing could have positive impact on the health of consumers, such as enhancing the bioavailability of essential dietary mineral and reduce the risk of kidney stones formation.

Molar ratio of phytate to Ca, Zn and Fe in cassava tuber flours

The molar ratio of phytate to minerals found for the seven cultivars of cassava tuber are presented in Table 2. The interaction between cultivars and processing effects were significant (P<0.05). Similarly, the effect of cultivars and processing methods on dependent variables were significant (P<0.05). The average calculated molar ratio of phytate to zinc was 48.89, 41.23, 38.44 and 24.52 for the raw tuber flour, boiled, sun-dried and fermented flours, respectively. The maximum and minimum values were found for unprocessed cultivar Koree (92.46 ± 0.06) and fermented cultivar 28 (0.51 ± 0.03) flours, respectively. The results show that the phytate: zinc molar ratio is above critical value (>1.5). As a measure of the bioavailability of zinc, we calculated the molar ratio of phytate x calcium to zinc. The average molar ratio of phytate x calcium to zinc of seven cassava roots cultivars

were 3.95, 1.88, 8.77 and 1.01 for raw tuber flour, boiled, sun-dried and fermented flours, respectively. The average value for sun-dried molar ratio was significantly (P<0.05) higher than for the raw tuber flour. This might be due to the reaction of Zn ion to other anti-nutrient factors in the flour. The highest and lowest molar ratio of phytate x calcium to zinc values were found for sun-dried cultivar 5538-19 (30.80 ± 0.03) and boiled cultivar 28 (0.02 ± 0.01), respectively. In addition to this, except for cultivars of 28 (raw, boiled & fermented=0.02); Hayik (boiled=0.28, sundried=0.31 & fermented = 0.30); 44/72-NW (fermented=0.30) and Koree (fermented=0.37) the phytate x calcium: zinc molar ratios are above the critical value (>0.5). The present study result shows limited Zn bioavailability in most of the seven-cassava tuber cultivars flour.

The molar ratio of phytate to iron in cassava tuber cultivars were calculated as 35.24, 30.52, 23.08 and 17.82 for the raw tuber flour, the boiled, sun-dried and fermented flours, respectively. Among the average values of processed flour, the molar ratio of phytate to Fe fermented flours were significantly (P<0.05) lower than others. The maximum and minimum molar ratio values were found for boiled cultivar Gamo (83.08 ± 0.01) and fermented cultivar Koree (0.12 ± 0.03), respectively. The phytate to iron molar ratio is found to be above critical value (>0.4). The bioavailability of iron is deficient and it should be advised to fortify cassava tuber foods with iron to overcome the shortage.

The molar ratio of phytate to calcium in seven cassava tuber cultivars were 0.22, 0.42, 0.22 and 0.23 for the raw tuber flour, the boiled, sun-dried and fermented cassava tuber flours, respectively. The effect of processing on molar ratio of phytate to calcium were found to be significant (P<0.05). The highest and the lowest values of phytate to calcium were found for the boiled cultivar 5538-19 (1.36 ± 0.02) and fermented cultivar 44/72-NW (0.07 ± 0.01) flours, respectively. Except for two boiled cultivars of 5538-19 (1.36) and 44/72-NW (0.59) all other values of phytate: calcium molar ratio are below the critical value (<0.5) indicating that the bioavailability of calcium in most of the seven cultivars of cassava tuber flours is satisfactory.

Table 1: Raw and processed cassava root cultivars oxalate to calcium molar ratio* (ox: ca) [b]

Treatments [a]	Cultivars				Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Marginal Mean	SD	% cv	PoC	PoPm	PoC x Pms
	28	192	5538-19	44/72-NW									
Raw	0.14 ± 0.00d	0.06 ± 0.00b	0.20 ± 0.01b	0.13 ± 0.00b	0.07 ± 0.00a	0.09 ± 0.00a	0.13 ± 0.00a	**0.12**	0.05	41.6	*	*	*
Boiled	0.08 ± 0.01b	0.04 ± 0.00a	0.41 ± 0.02d	0.45 ± 0.00d	0.07 ± 0.00a	0.15 ± 0.01c	0.13 ± 0.00a	**0.19**	0.17	89.4			
Sun-died	0.12 ± 0.01c	0.11 ± 0.00c	0.31 ± 0.01c	0.18 ± 0.01c	0.18 ± 0.00c	0.12 ± 0.00b	0.13 ± 0.00a	**0.16**	0.07	43.7			
Fermented	0.04 ± 0.01a	0.04 ± 0.01a	0.03 ± 0.00a	0.03 ± 0.00a	0.17 ± 0.00b	0.26 ± 0.00d	0.13 ± 0.00a	**0.10**	0.09	90.0			
Marginal means	**0.09**	**0.06**	**0.24**	**0.20**	**0.12**	**0.15**	**0.13**						
Critical value [p]	=2.5	=2.5	=2.5	=2.5	=2.5	=2.5	=2.5						

Values of molar ratio of the same column with different superscript letters are significantly different from each other with a<b<c<d (P<0.05),

[a] = methods of processing, [b] = results were mean values of triplicate determination, ***- Interactions significant at P<0.05,

* = mg of oxalate/MW of oxalate: mg of calcium/MW of calcium, cv- coefficient of variation, PoC - P of cultivar, PoPm - P of processing methods, PoC x Pms - P of cultivar X processing methods. [p] = Critical values were sourced from Hassan, et al.[19]

Table 2: Molar ratio of phytate to minerals {Phy.: Fe, Phy.: Zn, Phy.: Ca, [Ca]x[Phy.]: Zn}contents of seven cultivars of cassava roots flour[b]

Molar ratio	Treatment	Cultivars 28	192	5538-19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Marginal means	SD	% Cv	PoC	Po Pm	PoC x Pms
[ψ]Phytate/ Zn	Raw	0.52 ± 0.40b	58.47 ± 0.04c	0.46 ± 0.02a	44.73 ± 0.02c	85.00 ± 0.04c	92.46 ± 0.06d	60.58 ± 0.35c	**48.89**	38.82	79	*	*	*
	Boiled	0.51 ± 0.31a	51.99 ± 0.04a	1.10 ± 0.03c	63.84 ± 0.24d	89.70 ± 0.09d	69.52 ± 0.02c	11.94 ± 0.01a	**41.23**	36.29	88			
	Sun-dried	15.77 ± 0.44c	57.26 ± 0.07b	26.43 ± 0.16d	41.87 ± 0.90b	68.85 ± 0.18b	46.73 ± 0.03b	12.19 ± 0.18ab	**38.44**	21.24	55			
	Fermented	0.51 ± 0.03a	77.91 ± 0.13d	0.95 ± 0.02b	12.29 ± 0.01a	52.40 ± 0.01a	15.15 ± 0.06a	12.40 ± 0.03b	**24.52**	29.28	119			
Marginal means		**4.33**	**61.41**	**7.24**	**40.68**	**73.99**	**55.97**	**24.28**						
[e]Phytate/ Fe	Raw	44.59 ± 0.06c	49.96 ± 0.01d	0.40 ± 0.01a	36.16 ± 0.02d	70.97 ± 0.01c	1.17 ± 0.02d	43.42 ± 0.02d	**35.24**	25.89	73	*	*	*
	Boiled	47.90 ± 0.01d	38.05 ± 0.02b	0.57 ± 0.02c	33.09 ± 0.03c	83.08 ± 0.01d	0.52 ± 0.06c	10.41 ± 0.02a	**30.52**	29.80	97			
	Sun-dried	8.47 ± 0.09a	43.56 ± 0.03 c	14.18 ± 0.03d	32.44 ± 0.04b	51.78 ± 0.05a	0.38 ± 0.05b	10.74 ± 0.02b	**23.08**	19.54	84			
	Fermented	17.8 ± 0.13b	34.33 ± 0.03a	0.47 ± 0.03b	8.69 ± 0.03a	52.43 ± 0.01b	0.12 ± 0.03a	10.91 ± 0.03c	**17.82**	19.22	107			
Marginal means		**29.69**	**41.48**	**3.91**	**27.60**	**64.57**	**0.55**	**18.87**						
[Δ]Phytate/ Ca	Raw	0.22 ± 0.01d	0.14 ± 0.02b	0.40 ± 0.01b	0.17 ± 0.01b	0.19 ± 0.01a	0.33 ± 0.01d	0.18 ± 0.01b	**0.22**	0.10	45	*	*	*
	Boiled	0.19 ± 0.01c	0.12 ± 0.01a	1.36 ± 0.02d	0.59 ± 0.010d	0.31 ± 0.01b	0.27 ± 0.01c	0.08 ± 0.01a	**0.42**	0.45	107			
	Sun-dried	0.14 ± 0.01a	0.21 ± 0.01c	0.26 ± 0.01a	0.22 ± 0.01c	0.43 ± 0.01d	0.22 ± 0.01b	0.08 ± 0.01a	**0.22**	0.11	50			
	Fermented	0.17 ± 0.01b	0.38 ± 0.01d	0.41 ± 0.01c	0.07 ± 0.01a	0.42 ± 0.01c	0.09 ± 0.01a	0.08 ± 0.02a	**0.23**	0.16	69			
Marginal means		**0.18**	**0.61**	**0.20**	**0.26**	**0.34**	**0.23**	**0.11**						
[ω][Phy.]x[Ca]/Zn (Mol / Kg)	Raw	0.02 ± 0.01a	4.75 ± 0.06d	1.55 ± 0.30b	2.65 ± 0.03d	7.22 ± 0.05d	7.91 ± 0.03d	3.53 ± 0.02d	**3.95**	2.89	73	*	*	*
	Boiled	0.02 ± 0.00a	3.31 ± 0.09c	1.42 ± 0.02a	0.86 ± 0.06b	4.04 ± 0.07c	3.25 ± 0.06c	0.29 ± 0.02a	**1.88**	1.62	86			
	Sun-dried	22.90 ± 0.18b	2.34 ± 0.07b	30.80 ± 0.03d	1.56 ± 0.02c	1.74 ± 0.06b	1.72 ± 0.02b	0.31 ± 0.04c	**8.77**	12.57	143			
	Fermented	0.02 ± 0.01a	2.25 ± 0.02a	2.69 ± 0.05c	0.30 ± 0.01a	1.15 ± 0.03a	0.37 ± 0.02a	0.30 ± 0.02b	**1.01**	1.06	105			
Marginal means		**5.74**	**9.12**	**3.16**	**1.34**	**3.54**	**3.31**	**1.11**						

[b] results were mean values of triplicate determination (dwb) ± SD, values of respective molar ratio of the same column with different superscript, letters are significantly different from each other with a<b<c<d (P<0.05), [e] = mg of Phytate/MW of Phytate: mg of iron/MW of Iron, [ψ] = mg of Phytate/MW of Phytate: mg of Zinc/MW of Zn, [Δ] = mg of Phytate/MW of Phytate: mg of Calcium/MW of Calcium, [ω] = (mol/kg Calcium) x (mol/kg Phytate): (mol/kg Zn), PoC - P of cultivar, PoPm - P of processing methods, PoC x Pms-P of cultivar X processing methods, *- Interactions significant at P<0.05, cv-coefficient of variation.

Conclusion

All processing methods enhance the availability of nutrients in cassava tubers by decreasing the anti-nutritional factors (ANFs) including phytate and oxalate. Among the anti-nutritional factors analyzed, the low content of ANFs in the flour after three processing techniques of the seven cassava root cultivars is one good advantage for consumers of cassava in terms of inhibitory effect of ANFs nutrient availability. The three common processing techniques of cassava tubers results in substantial loss of minerals. Organized public information regarding removal of anti-nutrient factors by processing methods should be promoted.

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