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Effect of Fermentation on Some Minerals and Antinutrient Content of *Telfaira Occidentalis* and *Gnetum Africanum* Leaves

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ABSTRACT

Antinutrients are natural compounds in plant foods that interfare with the absorption of nutrients, thereby reducing there their bioavailability. Hence, the need to curb their effect in order to prevent deficiencies of micronutrients becomes paramount. This study therefore aimed at determining the effect of fermentation on some minerals and antinutrients content of Telfaira occidentalis and Gnetum africanum leaves. The vegetables were fermented in the open using deionized water. T. occidentalis and G. africanum leaves were fermented for 24 and 48 hours respectively. Mineral and antinutrient contents were determined using standard methods. The results showed that Fe, Zn, Ca, Cu and Mg content were significantly ($p<0.05$) higher in T. occidentalis than G. africanum, and fermentation significantly ($p<0.05$) increased the level of Zn, Ca, and Cu of both vegetable leaves, while iron (Fe) significantly ($p<0.05$) increased and Mg significantly ($p<0.05$) decreased in T. occidentalis, Fe significantly ($p<0.05$) decreased with Mg showing no significant ($p>0.05$) change in G. africanum. The antinutrient content of phytate and oxalate is significantly ($p<0.05$) higher in G. africanum and fermentation significantly ($p<0.05$) decreased these antinutrients in both vegetables. The study shows that fermentation significantly ($p<0.05$) increased the level of Zn, Ca and Cu, and significantly ($p<0.05$) decreased the phytate and oxalate content of both vegetables.

Keywords: *Vegetables, Fermentation, Antinutrients, Minerals.*

1.0 INTRODUCTION

Fermentation is one of the oldest and most economical methods of food production and preservation [1]. It is a process by which bacteria, yeasts and moulds convert sugars and carbohydrates into less complex products such as carbon dioxide and alcohol [2]. It involves the conversion of large molecules to small molecules or molecular oxidation/ reduction mechanisms mediated by selected microorganisms [3] Some studies have demonstrated that fermentation of legumes enhances their nutritive value and antioxidant properties; reduces some anti-nutritional endogenous compounds such as phytic acid, and exerts beneficial effects on protein digestibility and biological value [4, 1]

Telfairia occidentalis is a member of the family Cucurbitaceae commonly known as the fluted gourd, fluted pumpkin or ugwu which is widely cultivated in Nigeria. It has been reported to contain nutrients such as proteins, carbohydrates, vitamins, minerals and fiber. It also contains oxalates, saponins, glycosides, flavonoids, alkaloids and resins [5]. Its medicinal properties includes; antianeamic [6], antidiaebetic, hepatoprotective [7], and a purgative leafy vegetable [8].

Gnetum africanum is a dioecious plant belonging to the family Gnetaceae. It is a non-woody, wild vegetable that grows on trees and is commonly known in southeastern Nigeria as “Okazi” by the Igbo, and “Afang” by the Efik/Ibiobio. It is an important source of protein, essential amino acids and mineral elements [9]. Medicinally, it is used to treat nausea, sore throat and enlarged spleen and also used as a cathartic and as a poison antidote [10].

Leafy vegetables are commonly consumed especially when in season for their micronutrients and medicinal properties, however, they also contain antinutrients which interfare with the absorption of these nutrients. Application of fermentation technique has been shown to decrease the antinutrient content in some food products, but not much report is found in regards to leafy vegetables.

Therefore this research focused on determining the effect of fermentation on some minerals and antinutrient content of *Telfaira occidentalis* and *Gnetum africanum* leaves.

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The matured vegetables (*Telfaira occidentalis* and *Gnetum africanum*) were purchased from Sabon gari market in Zaria. The vegetables were authenticated at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria and a voucher number deposited, 23089 and 1259 for *Telfaira occidentalis* and *Gnetum africanum* leaves respectively.

2.2 Preparation of Plants

The harvested vegetables were washed, and divided into two parts, one part was air dried at room temperature and then grinded and the other part was fermented. Open fermentation was done, the vegetables were soaked in deionized water for 24 hours for *T. occidentalis* and 48 hours for *G.africanum* at room temperature for the inherent fermenting microorganisms and environmental fermenting microorganisms to act. The fermented vegetables was then be filtered out and oven dried, then grinded.

2.3 Determination of Mineral Content

Plant samples were digested using the method of [11]
Fe, Ca, Cu, Mg and Zn content of both leaves and their fermented derivatives was determined using the Atomic absorption spectrophotometry (AAS) method.

2.4 Determination of Phytate Content

Phytate content in both leaves was determined using the method of [12]

2.5 Determination of Oxalate Content

Oxalate content in both leaves was determined using the method of [13]

2.6 Determination of Phytate-Fe and Phytate-Zn Molar Ratios

The molar ratios of phytate to Fe and phytate to Zn in both vegetables and their fermented variants was calculated using the method of [14]

2.7 Statistical analysis

Results are presented as mean ± standard deviation except where otherwise stated. Data was analysed using statistical package for the social sciences (SPSS), version 20. Analysis of variance (ANOVA) was used to compare the means. The difference between the various means were compared using the Duncan Multiple Range Test. P values less than 0.05 ($p<0.05$) were taken as significant.

3.0 RESULTS

3.1 Mineral Content of Fermented and Unfermented *Telfaira occidentalis* and *Gnetum africanum* Leaves

The mineral content in unfermented and fermented *Telfaira occidentalis,* and *Gnetum africanum* leaves is shown in Table 1. *Telfaira occidentalis* shows significant ($p<0.05$) higher content of Fe, Ca, Cu, Zn and Mg than *Gnetum Africanum* leaves. Fermentation significantly ($p<0.05$) increased the level of Zn, Ca, and Cu of both vegetable leaves. Iron (Fe) significantly ($p<0.05$) increased while Mg significantly ($p<0.05$) decreased in *Telfaira Occidentalis*. In *Gnetum Africanum*, the level of Fe significantly ($p<0.05$) decreased while Mg shows no significant ($p>0.05$) increase.

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Table 1: Some Mineral Content of Fermented and Unfermented *Telfaira Occidentalis* and *Gnetum Africanum* Leaves

SAMPLE	***MINERAL ELEMENT (mg/100g)***				
	Fe	***Mg***	***Zn***	***Ca***	***Cu***
Telfaira occidentalis	77.63 ± 0.05^{c}	54.88 ± 0.60^{b}	7.28 ± 0.61^{c}	1029.77 ± 0.03^{b}	1.12 ± 0.02^{c}
***Telfaira occidentalis*, Fermented**	157.07 ± 0.14^{d}	44.43 ± 0.51^{a}	17.12 ± 0.06^{d}	1596.42 ± 0.25^{d}	1.21 ± 0.06^{d}
Gnetum africanum	21.33 ± 1.53^{b}	45.20 ± 0.63^{a}	1.40 ± 0.06^{a}	884.13 ± 0.25^{a}	0.30 ± 0.01^{a}
***Gnetum africanum*, Fermented**	20.49 ± 0.13^{a}	45.43 ± 0.51^{a}	3.58 ± 0.01^{b}	1316.42 ± 0.21^{c}	0.91 ± 0.08^{b}

Values are mean ± S.D of three determinations. Values with different superscript down the column differ significantly($p<0.05$)

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3.2 Some antinutrient content of Fermented and Unfermented *Telfaira occidentalis* and *Gnetum africanum* Leaves

Antinutrients content of unfermented and fermented *Telfaira occidentalis,* and *Gnetum africanum* leaves is presented in Figure 1 and 2. *Gnetum africanum* shows significant ($p<0.05$) higher levels of phytate and oxalate. There was a significant ($p<0.05$) reduction in the levels of phytate and oxalate for both vegetables after fermentation.

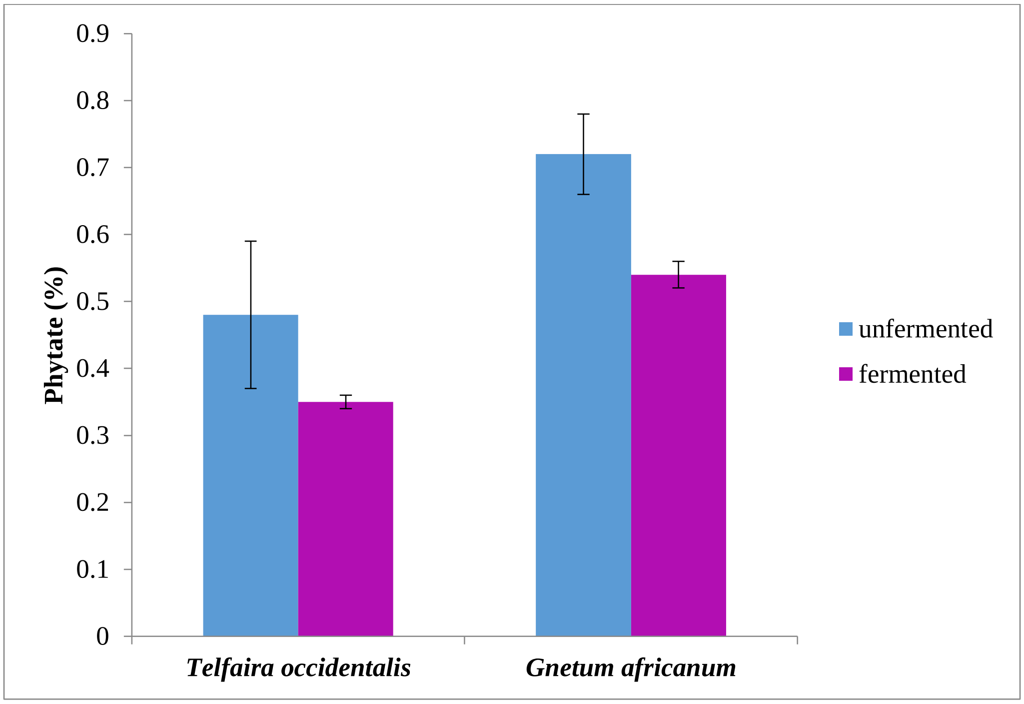

Figure 1. Phytate Content of Fermented and Unfermented *Telfaira occidentalis* and *Gnetum africanum* Leaves

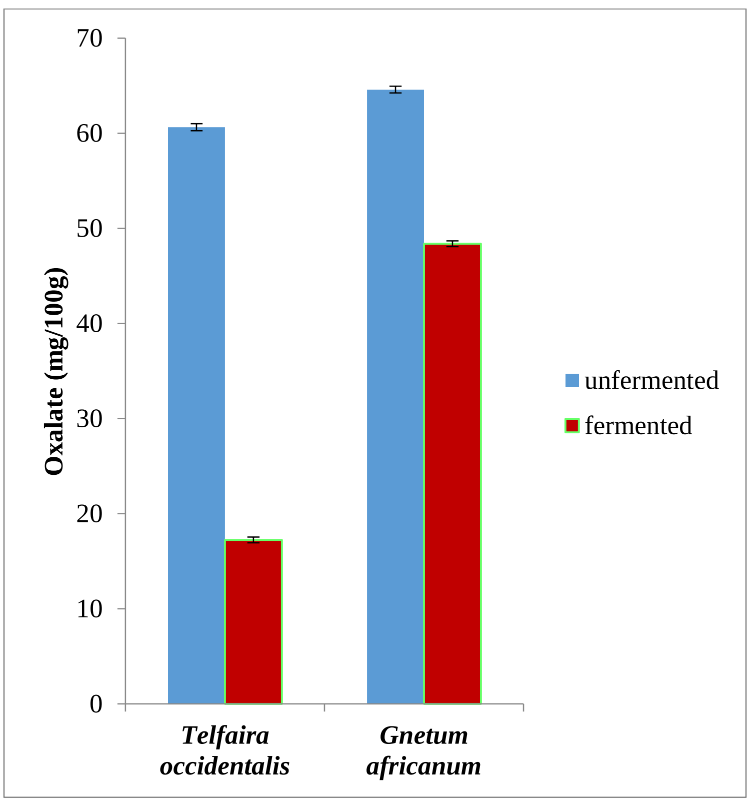

Figure 2: Oxalate Content of Fermented and Unfermented *Telfaira occidentalis* and *Gnetum africanum* Leaves

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3.3 Molar ratios of phytate:Fe and phytate:Zn in Fermented and Unfermented *Telfaira occidentalis* and *Gnetum africanum* Leaves

The molar ratios of phytate:Fe and phytate:Zn in Fermented and Unfermented *Telfaira occidentalis* and *Gnetum africanum* Leaves is shown in Table 2. Molar ratios for phytate-Fe and phytate-Zn were lower in *T.occidentalis* than *G.africanum* leaves. These ratios decreased in both leaves after fermentation.

Table 2: Molar Ratios of Phytate:Fe and Phytate:Zn in fermented and Unfermented *Telfaira occidentalis* and *Gnetum africanum* leaves

Samples	*Phytate:Fe*	*Phytate:Zn*
T.occidentalis, unfermented	*0.52*	*6.50*
T.occidentalis, fermented	*0.19*	*2.03*
G.africanum, unfermented	*2.85*	*50.97*
G.africanum, fermented	*2.20*	*14.95*

4.0 DISCUSSION

Minerals, classified as micronutrients are needed in small amounts. Deficiency in minerals, however, can have a major impact on health such as anemia and osteoporosis that commonly occur in both developed and developing countries [14] This study focused only on iron (Fe), zinc (Zn), copper (Cu), magnesium (Mg) and calcium (Ca).

Mineral contents were significantly ($p<0.05$) higher in *T. occidentalis* than *G.africanum* leaf. This is probably because of the ~~the~~ differences in botanical structure, as well as in the mineral composition of the soil in which the plants are cultivated. Other factors responsible for a variation in elemental content are preferential absorbability of the plant, use of fertilizers, irrigation water and climatological conditions [15, 16]. The major minerals analyzed were Ca and Mg, these are required in amounts greater than 100mg per day [17, 18]. Calcium is essential for healthy bones, teeth and blood [19, 20, 16]. The health of the muscles and nerves depends on calcium. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter acetylcholine, for the activation of enzymes such as the pancreatic lypase. It helps to regulate the activity of skeletal muscle, heart and many other tissues. Deficiency of calcium causes rickets, osteomalacia and scurvy. The recommended dietary allowance of Ca for children is between 500 and 1000 mg and 800 mg for adults [16]. Hence, both vegetables and their fermented variants can be used to combat Ca deficiency.

Magnesium (Mg) is a major mineral which is required for energy production, oxidative phosphorylation, and glycolysis. It contributes to the structural development of bone and is required for the synthesis of DNA, RNA, and the antioxidant glutathione. It also plays a role in the active transport of calcium and potassium ions across cell membranes, a process that is important to nerve impulse conduction, muscle contraction, and normal heart rhythm [21]. The recommended dietary allowance is 400-420mg in adult males and 310-320mg in adult females [22]. Both vegetables and there fermented derivatives are not rich sources of Mg since their contents are far below the recommended values.

The minor minerals analysed were Cu, Zn and Fe these are required in amounts less than 100mg per day [17, 18]. Copper has the main role in immune system, connective tissue and skeleton, blood formation, the blood vessels and nervous system. Zinc and copper are cofactors of the antioxidant enzyme, superoxide dismutase (Cu/Zn-SOD) which detoxifies the toxic superoxide radical [23, 24]. The daily dietary requirement for Cu is 0.9mg in adults, 1mg and 1.2mg in pregnant and lactating women respectively [25]. *T.occidentalis* leaves contain higher Cu contents which was significantly ($p<0.05$) higher than the

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requirement for adults, however the unfermented form meets the requirement during pregnancy and the fermented form meets the requirement for lactating women. The Cu content of *G.africanum* leaf is not a rich source of copper since its content is significantly($p<0.05$) lower than the Cu requirement for adults however, its fermented variant can be used as a rich source of copper. Zinc is necessary for the growth and multiplication of cells (enzymes responsible for DNA and RNA synthesis), for skin integrity, bone metabolism and functioning of taste and eyesight [26]. Zinc deficiency is characterized by recurrent infections, lack of immunity and poor growth. Growth retardation, male hypogonadism, skin changes, poor appetite and mental lethargy are some of the manifestations of chronically zinc-deficient human subjects [27]. The requirement for zinc is 1 mg/day in infancy;10 mg/day between 1 and 10 years of age, and 15 mg/day in adults. Pregnant and lactating women require 20 to 25 mg, while normal adults require 15 mg of zinc every day [16]. The unfermented variant of both plants could only meet the Zn requirement in infancy, fermentation significantly ($p<0.05$) increased the Zn content in both leaves, however, the increase in *G.africanum* did not meet the requirement for children and adults but that of *T. occidentalis* met the requirement, hence fermented *T. occidentalis* can be used to combat Zn deficiency.

Iron is an essential component of haemoglobin [28] and myoglobin [29]. It is necessary for growth, development, normal functioning and synthesis of some hormones and connective tissues [30, 29]. Its deficiency ranges from iron depletion to iron deficiency anaemia which affects numerous organs and systems [31]. Recommended intakes for children 4-8yrs is 10mg, 14-18yrs is 11mg (male), 15mg (female), for adults 19-50yrs is 8mg (males), 18mg (females), for those that are 51yrs and above is 8mg for both males and females [25]. Therefore, the Fe content of these vegetables and there fermented derivatives are good Fe sources.

Fe and Zn deficiencies are widespread among people in developing and developed countries [32]. Phytate to Fe molar ratio gives an index of satisfactory Fe absorbtion which should be less than 1 [33]. Similarly, phytate to Zn molar ratios greater than 15 is associated with Zn deficiency. The Phytate to Fe molar ratio was 0.52 and 0.19 in unfermented and fermented *T.occidentalis* leaves, 2.85 and 2.20 in unfermented and fermented *G.africanum* leaves. This shows that the level of phytate in *T.occidentalis* leaves was not high enough to impair Fe absorption but was high in *G.africanum* leaves, hence it might not meet up with dietary Fe requirements, this molar ratio can further be reduced by increasing the fermentation time of *G.africanum* leaf so as to increase the bioavailability of its Fe content.

The phytate Zn molar ratio was 6.50 and 2.03 in unfermented and fermented *T.occidentalis* leaves, and 50.97 and 14.95 in unfermented and fermented *G.africanum* leaves. This reveal that the Zn content in *T.occidentalis* leaves can meet up with dietary requirements probably because the phytate content was not high enough to impair Zn availability. The ratio was much higher in unfermented *G.africanum* leaf suggesting that it has higher phytate content, hence the need to increase the fermentation time.

Fermentation increased the iron content of *T. occidentalis* significantly ($p<0.05$) whereas it led to significant ($p<0.05$) decrease in *G.africanum*, there was a significant ($p<0.05$) decrease in Mg content of *T. occidentalis* and a non significant ($p>0.05$) increase in *G.africanum*. Zn, Ca and Cu increased significantly ($p<0.05$) in both vegetables The observed increase might be due to contribution by the fermenting microorganisms since the reduced antinutrients might have led to the release on some of the minerals bound to them, while the decrease might be due to leaching of the minerals into the fermentation medium probably due to the duration of fermentation. Similar changes in mineral content following fermentation were reported [34] where Fe and Zn content increased significantly after *Cassia obtusifolia* leaf was fermented. However, a significant decrease in the mineral contents of *T. occidentalis* leaves after fermentation for 5days [35], and also a significant decrease in mineral contents of *Sorghum bicolor* grains after fermentation for 3 days [36], have both been reported.

Antinutrients are chemicals which have been evolved by plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value [37].

Phytate, which is also known as inositolhexakisphosphate, is a phosphorus containing compound that binds with minerals and inhibits mineral absorption. The presence of phytate in foods has been associated with reduced mineral absorption due to the structure of phyate which has high density of negatively charged phosphate groups which form very stable complexes with

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mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+}causing non-availability for intestinal absorption [38]. They are generally found in food high in fibre especially in wheat bran, whole grains and legumes [39]. Phytate is hydrolysed via intermediate myo-inositolphosphates ($InsP_5$ to $InsP_1$) into myo-inositol and inorganic phosphate. Degradation of phytate can be achieved during food processing through the addition of exogenous phytases [40], by micro-organisms producing phytase or by endogenous cereal phytases [41]. Oxalate is an anti-nutrient which under normal conditions is confined to separate compartments. However, when it is digested, it comes into contact with the nutrients in the gastrointestinal tract [42] and binds with minerals, such as Ca^{2+}, Fe^{2+}, and Mg^{2+} rendering these minerals unavailable to the body.

The content of phytate and oxalate were significantly higher in *G.africanum* leaf than *T. occidentalis* leaf this is probably because phosphorus which is stored as phytate might be higher in *G.africanum* leaf and maybe there is a greater need for Ca regulation, protection against herbivory and plant survival in *G.africanum* leaf hence more oxalate was produced [43, 44]. There was a significant reduction in the antinutrient content of both vegetables after fermentation. The loss in phytic acid during fermentation was possibly due to the action of fermenting microorganisms which release phytase to hydrolyze phytate into inositol and orthophosphate [45]. Similar reduction level of phytic acid after fermentation was observed for fluted pumpkin seed and breadfruit seeds, respectively [46, 47]. Significant ($p<0.05$) reduction in phytate after fermentation of *Cassia obtusifolia* leaves were also reported [34]. The significant reduction in oxalate might be due to degradation by the fermenting micro-organisms, [48] which made them leach out of the vegetables due to the acidic medium created during fermentation.

5.0 CONCLUSION

This study has shown that fermentation significantly ($p<0.05$) increased the Zn, Ca and Cu, and significantly ($p<0.05$) decreased the phytate and oxalate content in both vegetables.

C0NFLICT OF INTEREST

The authors declare no conflict of interest

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