

## Influence of Tigernuts on the Antioxidant Vitamins Levels in Normal Weight Male Human Subjects

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### ABSTRACT

Tubers have been recognized as a valuable source of essential macro- and micronutrients vital for overall health. Among these, tigernut has been gaining popularity due to its rich composition. This tuber contains bioactive compounds such as sterols, alkaloids, tannins, saponins, resins, and vitamins E and C. Natural antioxidants have attracted significant attention from nutritionists, food manufacturers, medical professionals, and consumers due to their numerous health benefits. This study aimed to examine the effect of tigernuts on human vitamin A, C, and E levels in normal-weight male subjects under resting conditions, as well as to explore potential mechanisms of action. Methods: The study involved forty (40) male participants who were non-habitual tigernut consumers, aged 18–28 years. Anthropometric data were recorded for control subjects, who then rested for 1 hour and 30 minutes. Serum vitamin C levels were analyzed using a spectrophotometric method, with ascorbic acid showing maximum absorption at 478.5 nm in methanol. On a separate day, each participant was given 5g of tigernut to chew as a bolus, with the same subjects serving as both control and test participants. The findings revealed that tigernut consumption significantly increased ( $P < 0.05$ ) vitamin E levels from  $0.09573 \pm 0.021$  to  $0.8430 \pm 0.057$  mg/%. In contrast, vitamin A levels significantly decreased ( $P < 0.05$ ) from  $291.8 \pm 29.50$  to  $97.89 \pm 32.36$  mg/%. Vitamin C levels also declined post-consumption, from  $1.586 \pm 0.201$  to  $1.188 \pm 0.080$  mg/%, though this decrease was not statistically significant. The results suggest that tigernut consumption enhances vitamin E levels while reducing both vitamin A and C levels in normal-weight male subjects.

## INTRODUCTION

*Cyperus esculentus* L., commonly referred to as earthnut (chufa, earth almond), is a perennial tuberous plant belonging to the sedge family (*Cyperaceae*). It is widely cultivated in Brazil, Spain, East Africa, and several West African countries, including Nigeria [1]. In Ukraine, earthnut is grown in experimental and homestead plots. *Cyperus esculentus* L. is valued for its small, sweet, almond-shaped tubers, which serve as a source of edible oil [2]. These tubers, located at the plant's roots, possess a hard shell, a crisp texture, and a naturally sweet taste. The use of chufa tubers as a food source dates back to ancient times, owing to their high nutritional content. They contain 20–25% lipids, 20–35% starch, 12–28% sugars, and 5–9% proteins [3]. The oil extracted from these tubers finds applications in the food and processing industries, as well as in medicine. Given their biologically active compounds, earthnut tubers are considered promising for the development of dietary, children's, and specialized food products [2].

## LITERATURE REVIEW

This plant is extensively cultivated and consumed as a dietary supplement in the Arabian Peninsula and other regions. It is a fibrous-rooted, upright perennial that grows to heights of 1 to 3 feet and propagates via seeds and rhizomes [4]. The tubers of *Cyperus esculentus* (CE) are edible and have a characteristic sweet, nutty flavor. In Nigeria, fresh tubers are consumed in various ways, including roasting, baking, drying, eating raw, or processing into a beverage known as *kunnu* [5]. The fat content of *C. esculentus* is comparable to that of the olive plant, comprising 72% unsaturated fatty acids (oleic acid and linoleic acid) and 28% saturated fatty acids (palmitic acid and stearic acid). Extracts from *C. esculentus* have demonstrated anti-cancer, antimicrobial, anti-diarrheal, and anti-flatulence properties and have been used in treating anemia, urinary tract infections, and high cholesterol levels [4].

Beyond its role as a food source, the tubers of *Cyperus esculentus* have additional applications. In Spain, for example, they are utilized to produce a milk-like drink called *horchata* [6]. Tiger nut is highly nutritious, possessing a fat composition akin to that of olives, and is naturally gluten- and cholesterol-free. It is also one of the richest sources of flavonoids and contains significant amounts of water, fiber, alkaloids, digestible carbohydrates, saponins, and fatty oils (glycerides). Additionally, it provides essential minerals such as phosphorus, potassium, calcium, iron, zinc, magnesium, and manganese [7,8].

The oil extracted from *Cyperus esculentus* (tiger nut) is abundant in monounsaturated fatty acids, similar to the oils found in olives, avocados, and hazelnuts [9]. This monounsaturated oil contains high concentrations of unsaponifiable matter, phospholipids, and bioactive compounds, including tocopherols, phytosterols, and polyphenols. The small, round tubers growing along the roots have a mildly almond-like taste and can be consumed raw, cooked, or used to prepare the traditional drink *orxata* [10,11,12]. Research has also shown that tiger nut contains phytosterols, ascorbic acid (Vitamin C), tocopherol (Vitamin E), and  $\beta$ -carotene. These compounds, along with the unsaturated fatty acids present in tiger nut oil, may contribute to its overall antioxidant activity [13,14].

Various plants and their bioactive compounds have been identified for their potential in addressing health concerns. Certain medicinal plants, such as tiger nut (*Cyperus esculentus*), have demonstrated notable health benefits [15]. Approximately 160 phytochemical compounds from 101 plant species have been reported to offer potential liver and kidney protection. Even today, medicinal plants play a significant role in the treatment of liver and kidney disorders [16].

According to the World Health Organization, diabetes mellitus has evolved into a global non-communicable epidemic. It poses both medical and social challenges due to its rapid prevalence and the development of severe complications, such as angiopathies, which significantly impact quality of life and life expectancy [17]. Modern pharmacotherapy increasingly integrates traditional medicinal knowledge, particularly the use of phytopreparations [18]. Herbal remedies are being explored as monotherapy for preventing type 2 diabetes mellitus or as early-stage interventions for the disease. The search for new treatments for metabolic disorders linked to diabetes remains a high priority in both pharmacy and medicine. Phytotherapy is considered a promising alternative due to its advantages over synthetic drugs, including low toxicity, mild pharmacological effects, and suitability for prolonged use with minimal side effects [2]. There is growing public interest in identifying medicinal plants with a long history of use and fewer adverse effects. The primary aim of utilizing such plants is to manage metabolic disorders, as plant-derived metabolites closely resemble those of the human body [19]. In this context, the present study investigated the impact of *Cyperus esculentus* (CE) on antioxidant vitamin levels in normal-weight male subjects and explored potential mechanisms through which CE might influence these vitamin levels.

## **METHODOLOGY**

### **Subjects**

The study involved 40 normal-weight male volunteers (18-28 years) from Ambrose Alli University, who were non-habitual Tigernut chewers. Their health status was assessed via questionnaire and physical examination, with informed consent and ethical approval obtained.

### **Inclusion/Exclusion Criteria**

Subjects with hypertension, kidney, heart-related conditions, ulcers, diabetes, or other health issues were excluded. Normal-weight individuals (BMI 18.5-25.0 kg/m<sup>2</sup>) were studied, with age, weight, height, BMI, blood pressure, and heart rate recorded before the study.

### **Determination of Body Mass Index**

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m<sup>2</sup>). Subjects removed shoes and heavy clothing before measurement, with weight taken using a Camry balance (Italy) and height measured using a Henglida stadiometer (China). Individuals with a BMI of 18.5–24.9 were included.

Control subjects arrived at the laboratory fasting and rested before data collection. After recording anthropometric measurements, they relaxed for 1 hour 30 minutes before blood samples were taken for antioxidant vitamin

analysis. Serum vitamin C was measured spectrophotometrically at 478.5 nm in methanol, while vitamins E and A were analyzed separately, with  $\alpha$ -tocopherol absorbing maximally at 285 nm in methanol.

On a separate day, the same individuals acted as test subjects and consumed 5g of tigernuts as a bolus. They then drank 50ml of water to aid digestion. After resting for 1 hour 30 minutes, a second blood sample was collected for antioxidant vitamin analysis.

#### Collection of Blood Sample

A 7ml blood sample was drawn from the medial cubital vein using a vacutainer syringe on the same day as serum collection. The sample was transferred to an anticoagulant-free tube, allowed to clot for 60 minutes, and then centrifuged at 3,000 rpm for 10 minutes at room temperature to separate the serum.

#### Statistical Analysis

Statistical analyses were carried out using Graph Pad Prism Statistical Software version 8.1. The results were presented as Mean  $\pm$  SEM. A P-value of less than 0.05 was considered to be statistically significant.

### RESULT

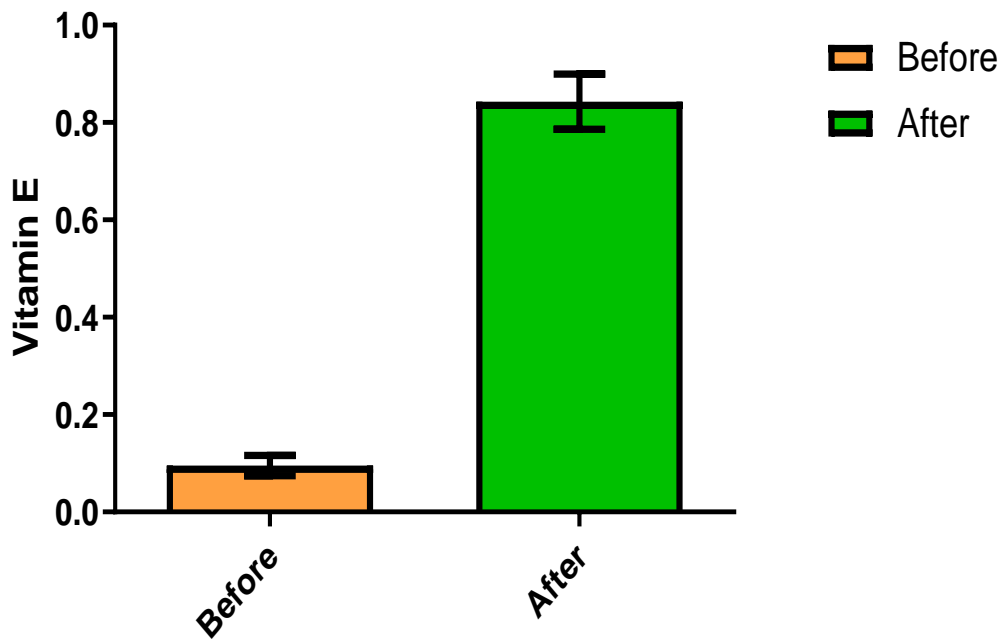


Fig I Showing the Effect of Tiger Nut Consumption on Vitamin E in Young Adult Individuals

There was a significant increase after consumption of tigernuts compared with before consumption

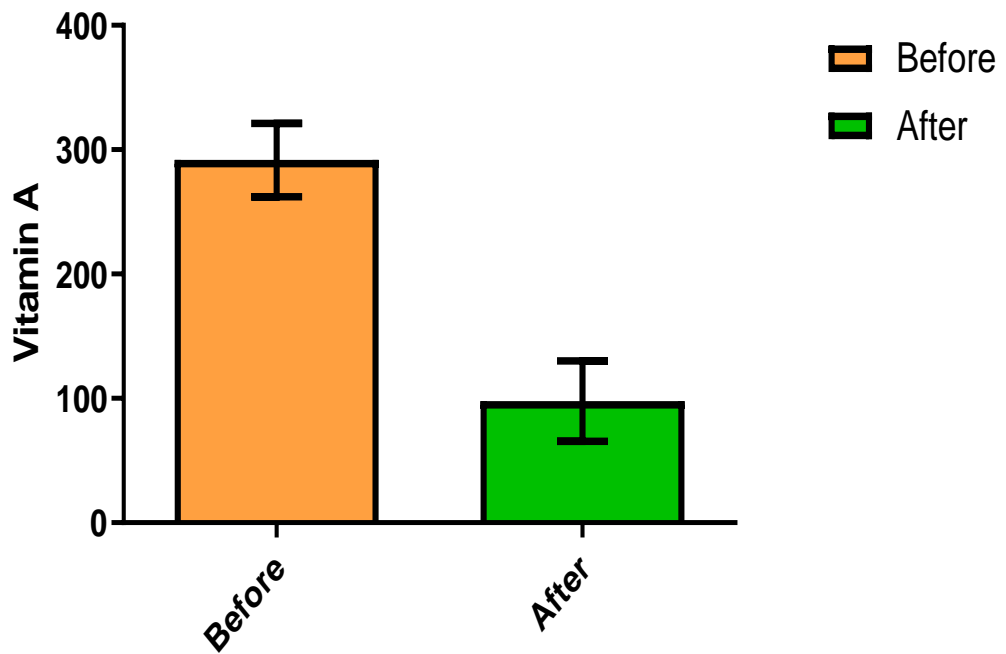


Fig II Showing the Effect of Tigernuts Consumption on Vitamin A in Young Adult Individuals

There was a significant decrease after consumption of Tiger nut compared with before consumption

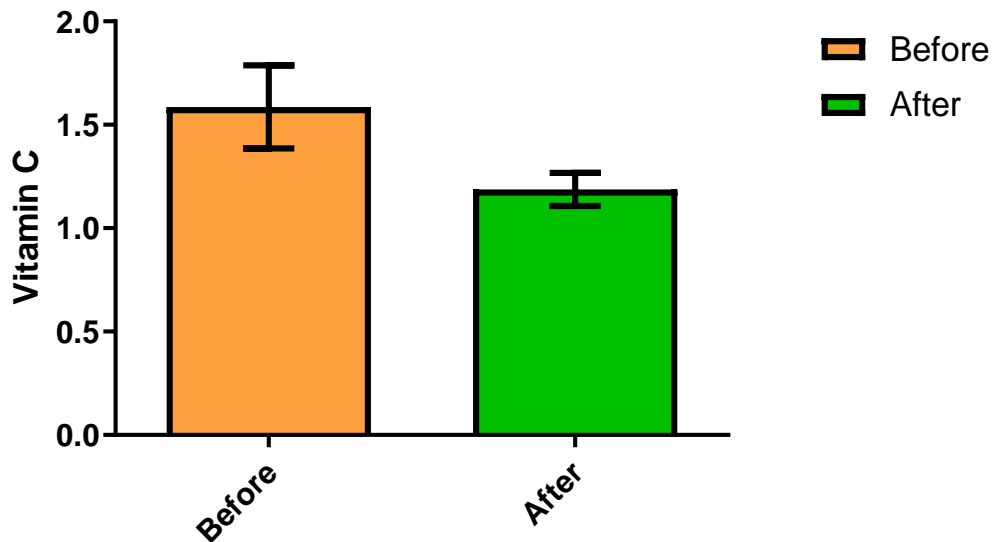


Fig III Showing the Effect of Tiger Nut Consumption on Vitamin C in Young Adult Individuals

There was no significant difference after consumption of tigernuts compared with before consumption

## DISCUSSION

The study explored the impact of tigernut consumption on the levels of Vitamins A, C, and E (antioxidant vitamins) in normal-weight male subjects at rest, as well as the potential mechanisms underlying these effects. The results showed a significant increase ( $P < 0.05$ ) in Vitamin E levels, which rose from  $0.09573 \pm 0.021$  to  $0.8430 \pm 0.057$  mg/%. In contrast, there was a significant decrease ( $P < 0.05$ ) in Vitamin A levels, dropping from  $291.8 \pm 29.50$  to  $97.89 \pm 32.36$  mg/%. Meanwhile, Vitamin C levels exhibited a non-significant decline following tigernut consumption, decreasing from  $1.586 \pm 0.201$  to  $1.188 \pm 0.080$  mg/ %.

Tigernuts contain various bioactive compounds, including sterols, alkaloids, tannins, saponins, resins, phenols, and *Vitamins A, B, C, D, and E*. The tuber is highly nutritious, comprising 22.14–44.92% lipids, 3.28–8.45% proteins, 23.21–48.12% starch, 8.26–15.47% fibers, and 1.60–2.60% ashes. Tigernuts also serve as a rich source of edible oils, particularly monounsaturated fatty acids, with a nutritional profile comparable to olive oil. Given its high lipid content (22.14–44.92%), the fatty acid composition of tigernut oil closely mirrors that of olive oil, which is widely regarded as one of the healthiest dietary fats. Additionally, tigernuts contain essential minerals such as sodium, potassium, calcium, iron, magnesium, zinc, copper, and phosphorus. The high antioxidant content of tigernut oil contributes to its superior oxidative stability compared to other vegetable oils.

The tuber also contains 77.49–80.01% essential fatty acids and 31.32–34.03 mg/100 g of essential amino acids. As a result, *Cyperus esculentus* oil aligns with medical recommendations that emphasize the benefits of consuming edible oils with more than 80% unsaturated fatty acids. Tigernuts are particularly rich in disaccharides, especially *D-sucrose*, which, when hydrolyzed, produces *D-glucose*, *D-galactose*, *D-xylose*, and *D-arabinose*. The tubers have been identified as a valuable source of essential macro- and micronutrients, including proteins, calcium, and *Vitamin C*, all of which are vital for human health. Tigernuts, in all their varieties, are rich in essential minerals required for metabolic processes. As a plant with potent antioxidant properties, tigernuts also contain *Vitamins A, C, D, and E*, among others.

Research conducted by [20] examined the antioxidant potential of different extracts from *Cyperus esculentus*. Both hexane and methanol extracts exhibited significant antioxidant activity, comparable to standard antioxidants such as *ascorbic acid*,  *$\alpha$ -tocopherol*, and *butylated hydroxyanisole (BHA)*. The observed free radical scavenging activity of tigernuts was attributed to the presence of phytochemicals such as flavonoids and phenolics. Similarly, a study on tigernut oil from Xinjiang, China, revealed that the oil possessed strong antioxidant and free radical scavenging properties in female experimental mice [21]. Growing interest in plant-based compounds for managing oxidative stress-related diseases underscores the need for further development of nutraceutical products derived from tigernuts and similar botanical sources, provided their efficacy is thoroughly validated.

Several factors may have contributed to the observed increase in *Vitamin E* levels. Among the antioxidant vitamins studied, *Vitamin E* likely exhibited the highest antioxidant activity. These findings are consistent with the research by [20], which linked the free radical scavenging potential of tigernuts to their phytochemical composition, including flavonoids and phenolics. Likewise, [21] reported that tigernut oil demonstrated notable antioxidant effects in female experimental mice, further supporting its potential role in counteracting oxidative stress. This suggests that tigernuts could be beneficial in the prevention and management of oxidative stress-related diseases.

Oxidative stress is a major contributor to the development of chronic diseases such as cancer, diabetes, and obesity [22]. Antioxidants play a crucial role in mitigating oxidative stress by neutralizing free radicals [23]. Given their health benefits, natural antioxidants are attracting considerable interest from nutritionists, food manufacturers, medical professionals, and consumers.

In addition to minerals, vitamins are essential for enzyme production, hormone synthesis, and various biological processes required for proper growth and development [24]. The vitamins present in *Cyperus esculentus* contribute to cell formation, tissue repair, and other critical metabolic functions. The significant increase in *Vitamin E* levels observed in this study may be linked to tigernuts' high vitamin content and the body's metabolic state during the experiment. Additionally, *Vitamin C* plays a crucial role in the synthesis of collagen, hormones, and neurotransmitters, suggesting a potential interaction between *Vitamin C* and *Vitamin E* in metabolic pathways.

Regarding *Vitamin C*, its high concentration in *Cyperus esculentus* may enhance its solubility and facilitate iron absorption, thereby boosting antioxidant activity. This process may contribute to the prevention of conditions such as coronary artery disease and cancer. However, the decline in *Vitamin C* levels observed in this study, though not statistically significant, suggests the possibility of a threshold effect, wherein excessive intake leads to a diminishing return.

*Tigernuts* are also rich in *Vitamin B1* (thiamine), which plays a key role in energy metabolism, and *Vitamin D*, which is essential for bone and muscle health. The association of *Vitamins D, B1, and C* with the absorption of minerals like phosphate, magnesium, and calcium further underscores their biological significance. Additionally, vitamins act as coenzymes in macronutrient metabolism, a role that may extend to *Vitamin E*. The importance of vitamins in maintaining overall health cannot be overstated, as they serve as an additional source of essential nutrients, including *Vitamin C, Vitamin B1, and Vitamin D*. Thiamine (B1) aids in regulating the central nervous system and helps the body adapt to stress, while *Vitamin E* contributes to cardiovascular health by preventing lipid oxidation, thereby reducing the risk of coronary artery disease.

As previously discussed, various factors influencing the levels of *Vitamins C and E* may have similarly affected *Vitamin A* levels. In this study, *Vitamin A* exhibited the weakest antioxidant activity, potentially due to lower scavenging capacity, a reduced physiological demand for *Vitamin A* at the time of the experiment, or an excess of *Vitamin A* prior to the study. Additionally, the way

tigernuts were processed may have influenced the observed changes in *Vitamin A* levels. These factors likely contributed to the significant reduction in *Vitamin A* levels observed in the study.

*Tigernuts* have numerous applications beyond direct consumption. They can be processed into juice for beverages or used in animal feed. Research suggests that processing or thermal treatment of plant materials can either increase, decrease, or have no effect on antioxidant capacity [25]. Consequently, the processing or thermal treatment of tigernuts may have influenced the antioxidant vitamin levels, particularly those of *Vitamins A, C, and E*.

## CONCLUSIONS AND RECOMMENDATIONS

In conclusion, tigernut consumption resulted in an increase in *Vitamin E* levels and a decrease in both *Vitamin A* and *Vitamin C* levels in normal-weight male subjects. The potential mechanisms underlying these effects have been explored in this study.

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### Conflict of Interest

The authors declare that we have no financial or personal relationship(s) which may have inappropriately influenced us in writing this paper.

### Author Contributions

1. Igbinovia, Edokpolor N. conceptualized the research, was involved in data curation and supervision of the research
2. Ohiwerei, Wisdom O. was involved in the formal analysis, investigation, methodology and reviewing of the manuscript
3. Ohiwerei, Faith O. was involved in investigation and review of the manuscript
4. Olugbenga, Mary A. was involved in validation of the research and writing of the initial manuscript
5. Festus, Oloruntoba O. was involved in the preparation of the initial manuscript and data visualization
6. Onokevbagbe, Elisha I. was involved in funding acquisition, providing essential materials and reagents for the research

**Data Availability Statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to patient confidentiality.

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