

## Assessment of vitamins and minerals of rats fed with plantain-maize pudding prepared using edible plant leaves and metallic plates

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### Abstract

Cooking materials are important factors that basically intended to provide covering, protection and consumers' choice of food products. The objective of the study was to determine the vitamin profile and minerals in the serum of rats fed maize-plantain pudding cooked using different leaves and metallic plates. Different maize-plantain pudding samples were prepared using plantain and maize flour blended together 1:1 ratio. In the animal study, thirty rats were divided into six groups of five rats each. Rats in Group A served as control. Group B rats were given Tween 80. Rats in Groups C, D, E and F were fed pudding prepared using plantain leaves, ginger leaves, aluminium plates and cast iron plates respectively. The rats in each group were allowed free access to feed and clean drinking water throughout the period of the experiment for 28 days. Mineral analysis was done using an atomic absorption spectrophotometer. Vitamin profile was determined using high performance liquid chromatography (HPLC). The results showed that serum minerals (calcium, potassium, magnesium, sodium, iron and phosphorus) and total vitamin content were significantly higher in rats fed with pudding prepared using ginger leaves when compared with plantain leaves, cast iron plate and aluminium plate. The serum iron content was significantly higher in rats fed pudding prepared using cast iron plate compared to other groups. Therefore, the use of locally accessible and consumable leaves and cast iron plates or utensils may be encouraged in diet preparation.

## 1. Introduction

Trends in the food industry have shown that cooking materials play an important role in the food production process. Besides the physical defence of the food product, cooking materials minimize the risk of spoilage (Kabuo *et al.*, 2015). Cooking materials are often used as a tool for the preservation and extension of shelf life by preventing or reducing water loss in fresh produce (Adejumo and Ola, 2008; Opara and Mditshwa, 2013). Cooking materials have evolved over the years beginning with the use of a variety of plant materials such as leaves and other containers such as polyethylene, plastics, glass and metals (Raheem, 2012).

However, the use of leaves as cooking material continues to be a common phenomenon in the traditional food industry in Nigeria and other African countries (Kabuo *et al.*, 2015; Omoike *et al.*, 2022). Supplements are molecules in food that all life forms have to require

to make energy, develop, create, and reproduce. These molecules in leaves and spices play a vital role in food products (George *et al.*, 2012, George *et al.*, 2015; George and Okpoghono, 2017; Okpoghono *et al.*, 2021) and prevention of disease conditions (Okpoghono *et al.*, 2018a, 2018b, 2018c, 2018d; Onakurhefe *et al.*, 2020; Okonta *et al.*, 2021). Leave packaged foods are not only available in rural areas alone as they are commonly found in large restaurants in urban areas as well. These foods have gained widespread acceptance among the elites who not only consider them exotic but also antioxidant and flavour-enhancing (Ojekale *et al.*, 2007; George *et al.*, 2019; Ugwu *et al.*, 2021; Ejueyitsi *et al.*, 2022, 2023; Okpoghono, Omoriare, Igue *et al.*, 2023, Okpoghono, Osioma, Metie *et al.*, 2023). Leaves commonly used for wrapping food include those of *Musa paradisiaca* (plantain), *Musa sapientum* (banana), *Xanthosoma sagittifolium* (cocoyam), *Tectona grandis*, *Thespesia populnea*, *Cola nitida* and sheaths of *Zea*

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*mays* (maize). These leaves are used to either wrap the food prior to cooking or just after cooking until they are consumed. There has been no information regarding *Zingiber officinale* (Ginger) leaves used for wrapping food like other leaves. The leaves of *Z. officinale* are sliced or chopped and added raw to salads.

Foods such as “moi-moi” (bean pudding) and “epiti” (steamed maize-plantain pudding) are among the foods that are commonly wrapped with different leaves in Nigeria. Recently, there seems to be an increased consumer preference for the consumption of epiti cooked using different leaves and metallic plates as observed in most cities in Nigeria, particularly in the Niger Delta region. Cooking with cast iron can intensify the iron content of foods. Several studies have shown that iron can be released into foods that are cooked in iron cookware (Otuaga et al., 2020a, 2020b). This may help to reduce iron deficiency (Jain, 2018). Aluminium cookware, not together with other sources of dietary aluminium, is well-thought-out to be a potential source of this metal to human beings (Al Zubaidy et al., 2011). Studies have shown that the aluminium level in food is great, and food cooked with aluminium utensils has a higher aluminium content which can be detrimental to healthy individuals and particularly to patients with chronic renal failure (Al Zubaidy et al., 2011).

Information abounds in literature on the vitamins and minerals properties of many foods and this has promoted their life use internationally as natural preservations and components of foods to promote good health. There is information on the ingredients used in cooking our indigenous foods, however, the materials used in regard to their preparations and the level of consumption are not well documented. There is also little knowledge of the vitamin profile and minerals of maize-plantain pudding commonly consumed locally. It is therefore necessary to evaluate the vitamins and minerals of this diet cooked using different edible leaves and metallic plates/utensils to diversify their uses. The results that will be obtained from the study may help to ascertain which of the pudding cooked using different edible leaves and metallic plates has a higher amount of vitamins and minerals. In view of the significant role nutritional practices play on the diet and health of individuals, this study was conducted to assess the intake of vitamins and minerals from maize-plantain pudding recipes, using experimental rats as the unit of analysis. The maize-plantain pudding, with information on the vitamins and minerals properties, due to the dietary nutrients, would attract recognition internationally. This would create employment for Africans chiefly Nigerians who would like to propagate and process the maize-plantain pudding recipes.

## 2. Materials and methods

### 2.1 Materials

Plantain fingers and dried maize were purchased from Amai local market, in Delta State. The aluminium plate and cast-iron plate were purchased from the Obiaruku market. Plantain leaves (*Musa paradisiaca*) and ginger leaves (*Zingiber officinale*) were obtained from a local farm in Amai. All the chemicals that were used for this study were of analytical grade.

### 2.2 Plantain and maize flour preparation

Unripe plantain fingers were washed in distilled water, peeled and each finger was cut into halves. All the peeled halves were boiled for 10 mins in distilled water in the cast iron cooking pot. The cooked samples were sliced (1.5 mm) and sun-dried (as it has been processed locally). The dried plantain and maize were ground to powder (flour sample) using a Warring blender. The samples were filtered with a sieve that has an aperture size of 425 µm. The plantain and maize flour were blended in 1:1 ratio. The blended sample was used for the preparation of pudding.

### 2.3 Maize-plantain pudding preparation

Ngoddy et al. (1986) recipe method was used for maize-plantain pudding formulation. Ingredients for the formulation were as follows: plantain/maize flour blend (300 g), ground tatashe pepper (*Capsicum annum*) (60 g), onion (*Allium cepa*) (60 g), salt (20 g), hot water (70°C) 900 mL, Maggi cube (8 g), and vegetable oil (140 mL). The food items were added together in a mixing bowl using a wooden handle to form a smooth paste. The paste was allowed to stand for 3 mins and mixed thoroughly. After that, 300 mL of the paste was dispensed into the aluminium plate and cast iron plate and then wrapped with two different edible leaves (plantain leaf: *M. paradisiaca* and ginger leaf: *Z. officinale*). This was allowed to steam for 1 hr using a modern cast iron pot. The products were allowed to cool and then dried in an electric oven (40°C) for further analysis.

### 2.4 Preparation of sample extract

The maize-plantain pudding samples were dried in an electric oven (40°C). Approximately 5 g of each dried sample were homogenized in 45 mL aqueous tween 80 (5% tween 80) using laboratory mortar and pestle to dissolve the oil present in the samples. The samples were filtered into sample bottles with clean muslin cloth and kept in the refrigerator at -4°C for further analysis. *In vitro* study (determination of minerals, vitamin E and beta carotene) was carried out on the extract of raw pudding, cooked pudding wrapped with ginger leaves

and plantain leaves, and cooked pudding prepared using cast iron and aluminium plates.

### 2.5 Experimental animals

Mature male albino Wistar rats (126) weighing 120-140 g were obtained from the animal house of the Department of Anatomy, Delta State University, Abraka. The experimental rats were housed in clean plastic cages and left to acclimatize for one week on grower's mash. After the acclimatization period, the rats were weighed and their weights were recorded for dosage determination.

### 2.6 Determination of dosage ( $LD_{50}$ )

Approximately ninety-six rats were used for dosage determination. This was done using the method of Karber's as described by Chinedu *et al.* (2013). The rats were divided into six groups of four per group for each extract. Control experimental rats were not given any sample extracts. The pudding sample extracts were administered separately to all four rats in each group starting with a single dose of 100 mg/kg b.wt. Animals were observed for a period of 1 hr, occasionally for 3 hrs for the severity of any toxic signs and mortality. If no mortality is observed at this dose, the same procedure was repeated for dose levels of 200 mg/kg b.wt, 400 mg/kg b.wt, 800 mg/kg b.wt and 1600 mg/kg b.wt. The  $LD_{50}$  was thus determined, which was selected for the protective animals study. The rats were observed up to 3 days after administration to find out for any delayed mortality. No mortality was observed in rats given the different pudding samples (aqueous extract of pudding prepared using plantain leaves, ginger leaves, aluminium plates and cast iron plates) from 100 - 1600 mg/kg body weight. The  $LD_{50}$  of the sample were greater than 1600 mg/kg body weight and hence has a high degree of safety. However, 400 mg/kg body weight was chosen for the protective animal study due to the positive behavioural signs and changes of the rats given this dose.

### 2.7 Treatment of animals

Thirty rats were divided into six groups per five rats. The rats in each group were allowed free access to clean drinking water throughout the experiment for 28 days.

Group A: control (rats were not given pudding aqueous extract and Tween 80).

Group B: Tween 80 (rats were administered Tween 80 only).

Group C: plantain leaves pudding aqueous extract (rats were administered aqueous extract of maize-plantain pudding prepared using plantain leaves).

Group D: ginger leaves pudding aqueous extract (rats

were administered aqueous extract of maize-plantain pudding prepared using ginger leaves).

Group E: aluminium plates pudding aqueous extract (rats were fed aqueous extract of maize-plantain pudding cooked using aluminium plate)

Group F: cast iron plates pudding aqueous extract (rats were given aqueous extract of maize-plantain pudding cooked using cast iron plate).

### 2.8 Blood collection and preparation of serum

The rats were sacrificed on the 29<sup>th</sup> day. The blood was collected by cardiac puncture using a hypodermic syringe and needle and then transferred to an anticoagulant-free test tube. Afterwards, the clotted blood was centrifuged at  $2,500\times g$  for 15 mins to separate the serum which was stored in the refrigerator, for further biochemical analysis.

### 2.9 Biochemical analysis

#### 2.9.1 $\alpha$ -tocopherol (vitamin E)

$\alpha$ -Tocopherol content was determined using the method described by Kivcak and Mert (2001). The volume of 20  $\mu$ L-100  $\mu$ L of  $\alpha$ -tocopherol and 40  $\mu$ L of the sample was used for the estimation. The volume was made up to 3 mL using chloroform, 1 mL of 2, 2-dipyridyl, and 1 mL of  $FeCl_3$  solution. The mixture was incubated at 37°C for 15 mins, and the absorbance of the reaction mixture was read at 520 nm.

#### 2.9.2 $\beta$ -carotene

Carotene content in the samples was analysed by using the Malaysian Palm Oil Board (MPOB) test method described by Dauqan *et al.* (2011). About 50  $\mu$ L sample was dissolved with 2 mL n-hexane. The solution was transferred into a 1 cm quartz cuvette and the absorbance was measured at 446 nm against n-hexane. The carotene content of different samples was calculated as  $\beta$ -carotene in parts per million (ppm).

#### 2.9.3 Determination of vitamins

The vitamins in the extract were determined using high performance liquid chromatography (HPLC). The analyses were carried out using the method described by Seal (2016). The mobile phase comprises 1% aqueous acetic acid solution (Solvent X) and acetonitrile (Solvent Y), and 2 mL/min was adjusted as the flow rate. The column was controlled at 28°C and the sample injection volume was 5  $\mu$ L. The HPLC chromatograms were detected using a photodiode array UV detector at wavelengths: 200-400 nm. The compound was identified based on their retention time and by confounding with the standards under the same conditions. Sample quantification was done by measuring the integrated

peak area.

### 2.9.4 Mineral analysis

Mineral analysis was done according to the method described by Onwuka (2005) using an atomic absorption spectrophotometer.

### 2.10 Statistical analysis

All the results were expressed in mean bars, mean  $\pm$  SD. The results were analyzed using Analysis of variance (ANOVA). A significant difference between means was determined at  $P < 0.05$  confidence level using the least significant difference (LSD).

## 3. Results and discussion

### 3.1 Changes in vitamin profile

Vitamins are varied compounds in structure, biological actions and chemical properties and are essential for metabolism function. The results of vitamin E and  $\beta$ -carotene of pudding wrapped with different leaves, aluminium and cast iron plates (in-vitro) are presented in Table 1. In the present study, a significant decrease was observed in vitamin E and  $\beta$ -carotene levels of CPGL, CPPL, CPCIP and CPAP when compared to RP. However, the vitamin E and beta-carotene of CPGL and CPPL were significantly higher when compared to CPCIP and CPAP. The high level of vitamin E and  $\beta$ -carotene in samples could be invariably attributed to the use of crude red palm oil, which is one of the richest sources of  $\beta$ -carotene for the preparation of these dishes. Important health benefits have been attributed to carotenoids. Some carotenoids, such as  $\beta$ -carotene, are capable of being converted into vitamin A, thereby playing an important nutritional role (Dauqan *et al.*, 2011). HPLC vitamin profiles in the serum of rats are shown in Table 2. Vitamin D, E, K, B6 and biotin levels were higher in the serum of rats given pudding samples in comparison to the control. Although vitamins were in trace quantities in the serum, they have very essential roles to play in human health. However, the trends of total vitamin concentrations in the serum of the entire group using HPLC (Figure 1) were as follows; rats administered pudding prepared using ginger leaves

(1683.79 ppm) > rats fed with pudding prepared using plantain leaves (1501.61 ppm) > rats given pudding cooked using cast iron plate (1276.97 ppm) > rats fed with pudding cooked using aluminium plate (1108.32 ppm) > rats administered Tween 80 only (775.09 ppm) > rats not given pudding aqueous extract and Tween 80 (control) (765.19 ppm). The low serum total vitamin concentrations in rats fed pudding prepared using aluminium plate may be due to the leaching of aluminium into the prepared pudding, which could instigate aluminium toxicity. Concurrently, Al toxicity inhibits the uptake of water and nutrients. Several reports have provided indications that toxic  $Al^{3+}$  alters nutrients (Boran *et al.*, 2019).

Table 1. Vitamin E and Beta-carotene of pudding wrapped with different leaves, aluminium and cast iron plates (in-vitro).

Samples	Vitamin E (mg/g)	Beta-Carotene (ppm)
RP	59.40 $\pm$ 7.12 <sup>a</sup>	100.50 $\pm$ 19.10 <sup>a</sup>
CPGL	34.50 $\pm$ 5.20 <sup>b</sup>	88.60 $\pm$ 12.45 <sup>b</sup>
CPPL	28.50 $\pm$ 6.20 <sup>c</sup>	76.20 $\pm$ 8.10 <sup>c</sup>
CPAP	18.80 $\pm$ 3.25 <sup>d</sup>	65.20 $\pm$ 10.65 <sup>d</sup>
CPCIP	23.50 $\pm$ 5.20 <sup>c</sup>	71.10 $\pm$ 14.90 <sup>c</sup>

Values are presented as mean $\pm$ SD of triplicates. Values with different superscripts within the same column are significantly different ( $P < 0.05$ ). RP: Raw pudding, CPGL: cooked pudding wrapped with ginger leaves, CPPL: cooked pudding wrapped with plantain leaves, CPCIP: cooked pudding prepared using cast iron plate and CPAP: cooked pudding prepared using aluminium plate.

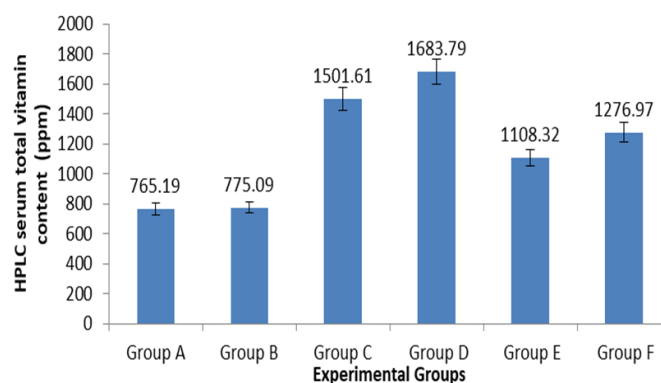


Figure 1. HPLC total vitamin content in the serum of rats fed aqueous extract of maize-plantain pudding prepared using different leaves and metallic plates.

Table 2. HPLC vitamin profile in the serum of rats.

Component (ppm)	Serum vitamin profile				
	Group A	Group B	Group C	Group D	Group E
Vitamin D (Cholecalciferol)	13.467	13.467	42.011	47.640	35.187
Vitamin E (Tocopherol)	705.433	715.432	1099.917	1143.697	970.836
Vitamin K (Phylloquinone)	12.398	12.398	160.053	184.837	67.641
Vitamin B6 (Pyridoxine)	15.259	15.259	183.015	260.752	22.993
Biotin	18.634	18.534	16.614	46.869	11.661
Total	765.191	775.090	1501.610	1683.794	1108.319

Vitamin E is a very important antioxidants which protect the cell membranes from oxidative stress/damage caused by free radicals. Vitamin D possesses an antioxidant property and is required for the maintenance of normal connective tissues, and wound healing and also facilitates the absorption of dietary iron from the intestine. This justifies the improvement of the antioxidant activity of the pudding. Deficiencies of these vitamins may prompt the red cell membranes to damage leading to haemolysis (Adesina, 1982). Riboflavin and niacin are necessary for oxidative phosphorylation and for coenzyme formation respectively (Adesina, 1982). The body needs biotin to metabolize carbohydrates, fats, and amino acids, the building squares of protein. Biotin is frequently prescribed for reinforcing hair and nails (Shea et al., 2021). Vitamin K makes an alteration to form different proteins that are required for blood clotting and the building of bones. Prothrombin may be a vitamin K-dependent protein straightforwardly included with blood clotting. Osteocalcin is another protein that requires vitamin K to deliver sound bone tissue (Shea et al., 2021).

3.2 Changes in minerals content

The mineral content of the raw and cooked pudding (*in vitro*) is presented in Figure 2. The significant decrease observed in calcium, potassium, magnesium and sodium levels of CPGL, CPPL, CPCIP and CPAP when compared to RP could be due to the effect of temperature on the cooked food sample (Hosseini et al., 2014). The high potassium and magnesium levels observed in pudding could be due to the plantain and maize which serves as the major ingredient in the preparation of the pudding. The high calcium content

could have been a result of the ingredients such as pepper, maize, maggi and onion which are sources of calcium. In Figure 3, there were significant differences ( $P<0.05$ ) in the serum mineral content of the rats fed with maize-plantain pudding wrapped with different leaves for all the mineral elements assessed. Serum potassium was the highest in rats fed aqueous extract of maize-plantain pudding wrapped with plantain and ginger leaves in comparison to the other experimental groups. The differences could be a result of differences in the mineral content of the various leaves which may have leached into the pudding samples. Plantain leaves have been reported to contain high amounts of potassium and calcium (Okareh et al., 2015). This may further provide another source of obtaining potassium in food as well as value addition for plantain leaves. The highest content of potassium, magnesium and sodium were obtained in pudding wrapped with ginger leaves and were significantly different ( $P<0.05$ ) from other samples. This implies that ginger may complement other sources of the minerals for the normal functioning of the body. Magnesium and sodium are components of the electrolyte which in synergy help to maintain the body cells and regulation. Previous study by Ayo-Omogie et al. (2010) reported that minerals such as potassium, magnesium and phosphorus constitute a greater percentage of the total mineral contribution in plantain. The decrease in minerals of rats fed pudding prepared using an aluminium plate could have been due to aluminium inhibitory properties on mineral absorption. A study has shown that aluminium may cause inhibition of intestinal absorption of phosphorous and this may be shadowed by an increase in calcium loss (Rahimzadeh et al., 2022).

The serum iron content was significantly ( $P<0.05$ ) high in rats fed with aqueous extract of maize-plantain pudding cooked using a cast iron plate. This could be a result of leached iron from the cast iron plate into the pudding samples. However, there are indications that

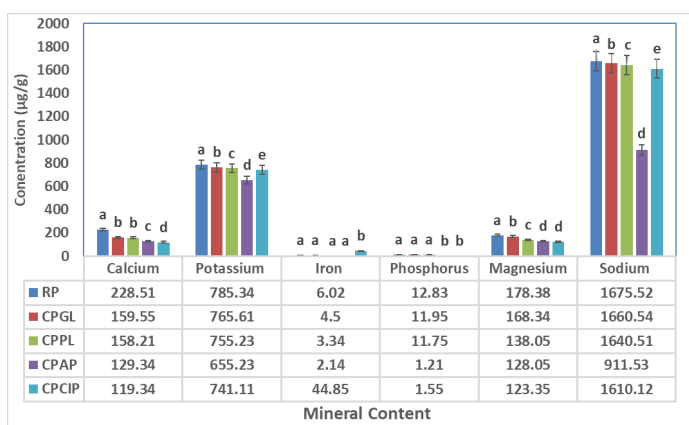


Figure 2. Mineral content of cooked pudding wrapped with edible plant leaves, aluminium and cast iron plates (*in vitro*). Data presented as means of triplicate determinations. Bars with different notations within the same mineral content are statistically significantly different ( $P<0.05$ ). RP: Raw pudding, CPGL: cooked pudding wrapped with ginger leaves, CPPL: cooked pudding wrapped with plantain leaves, CPCIP: cooked pudding prepared using cast iron plate and CPAP: cooked pudding prepared using aluminium plate.

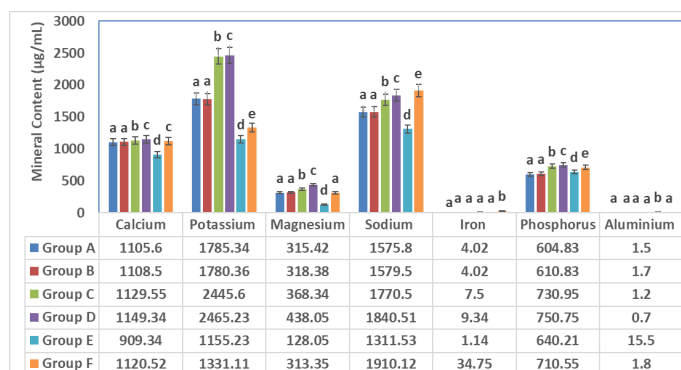


Figure 3. Serum mineral content of rats fed prepared pudding wrapped with different leaves, aluminium and cast iron plates (*in vivo*). Data presented as means of triplicate determinations. Bars with different notations within the same mineral content are statistically significantly different ( $P<0.05$ ).

iron-containing cookware could serve as a means of reducing iron deficiency and iron deficiency anaemia (IDA), especially among children (Alves et al., 2019). The possible advantages of iron-containing cookware include virtual cost-effectiveness and complementary blend with other interventions (Alves et al., 2019; Otuga et al., 2020b). The decrease in serum iron content of rats fed pudding prepared using aluminium plate (Figure 3) could have been due to the similarity of ionic radii of  $Al^{3+}$  and  $Fe^{3+}$ , there is the likelihood of  $Al^{3+}$  appearing in  $Fe^{3+}$  sites (El-Sayed et al., 2011). Consequently, aluminium is bound to  $Fe^{3+}$  transporting protein. This mechanism may decrease the binding of  $Fe^{2+}$ , augment free intracellular  $Fe^{2+}$ , and result in the peroxidation of membrane lipids leading to membrane damage (El-Sayed et al., 2011; Ekakitie et al., 2021).

#### 4. Conclusion

The current study presents new information on pudding preparation using edible plant leaves and metallic plates. The findings show that the serum minerals and vitamins content were significantly higher in rats fed pudding prepared using ginger leaves compared to plantain leaves, cast iron and aluminium plates. The variations in the vitamins and minerals content of the pudding products suggest that the different leaves may have impacted metabolites in the samples. Serum iron was expressively high in rats fed with pudding prepared using cast iron compared to ginger leaves, plantain leaves and aluminium plates. Therefore, the use of locally available and consumable leaves and cast iron plates or utensils for pudding preparation or diet should be invigorated.

#### Conflict of interests

The authors declare no conflict of interests.

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