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Bioavailability of Mineral Nutrients in Plantain Based Product Enriched With Bambara Groundnut Protein Concentrate

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Abstract

Plantain based products (amala, cookies, bread and cakes) enriched with bambara groundnut protein concentrate were produced. Total minerals content of the products were investigated using the atomic absorption spectrophotometry. A total of six essential minerals: iron, calcium, potassium, phosphorus, magnesium and sodium were determined. The bioavailability of these minerals was studied using pepsin and pancreatin enzymes in-vitro digestion method. The products developed were high in potassium, iron and phosphorous and low in sodium. However not all the minerals detected were bioavailable. Only 59.7% and 68.1% of potassium were available in soluble forms in bread and 'amala', respectively while 60.0% and 64.8% of iron were released in soluble forms in 'amala' and cookies and these values are significantly higher, compared to 53.0% Fe and 50.2% Fe released as soluble fractions in bread and cakes, respectively. The Ca/P ratio for cakes and bread are 1.1 and 0.8, respectively which is significantly higher ($P > 0.05$) compared to Ca/P ratio for 'amala' (0.4) and cookies (0.5). Therefore, the developed cakes and bread can serve as good sources of calcium and phosphorous which are considered essential for bone formation and teeth development in children.

Keywords: plantain products, protein concentrate, minerals, bioavailability

1. Introduction

Plantain is a major food crop in the humid and sub-humid parts of Africa and a major source of energy for millions of people in these regions (Aseidu et al., 1992). Plantain had been known to contain some micronutrients. Adeniji et al. (2006) determined micronutrients of some plantain varieties; and reported that Agbagba – a local variety had the highest iron content (36.5 µg/g), followed by FHIA 17 (16.135 µg/g). These values are higher compared to earlier report on raw green plantain by Ahenkora et al. (1996). In Rivers State and some parts of Nigeria, nursing mothers are encouraged to eat cooked pounded plantain mixed with red palm oil and taken with fresh fish soup in the first three months after delivery due to the belief that plantain iron aids blood regeneration and circulation. In Nigeria, 50% of children and 61% of women in the southeast region, suffer from chronic anemia due to iron deficiency (FGN/UNICEF 1994), whose symptoms includes learning disability, mental retardation, poor physical development and reduced ability to fight infectious diseases, ultimately leading to premature death. It has been reported by Adeniji et al. (2006) that the new plantain and banana hybrids contributed much higher levels of iron in human diet compared to 0.2-0.3 mg/100 g values obtained from two varieties of sweet potato. Other minerals such as potassium, calcium, phosphorus, manganese, sodium and copper had been reported in plantain by several researchers (Izonfou & Omauru, 1988; Baiyeri, 2000; Ogazi, 1984; Adeniji et al., 2007). However, information on the bioavailability of these minerals in plantain and plantain based product is scanty. Humans need many kinds of essential minerals throughout life. Suzuki and Wada (1994) reported that sixteen or more kinds of minerals are essential in human nutrition; and each essential mineral has many diverse physiological functions. The evaluation of diets for the adequacy of minerals requires the knowledge of both the amount and the bioavailability for intestinal absorption of minerals. Yasumoto (1994) observed that although minerals are widely distributed in foods, variation in the bioavailability of minerals was found among foods and concluded that bioavailability of food minerals can be affected by various factors such as proteins, phytic acid; other minerals and dietary fibre. Oral ingestion is the only natural path to supply humans with trace elements and other essential nutrients which are indispensable for growth, normal physiological functioning and the maintenance of life. Bioavailability (bio accessibility) is the maximum amount of mineral released from the matrix during gastro intestinal digestion that becomes available for intestinal absorption (Oomen et al., 2002). Digestion using simulated gastric and intestinal fluids provides valuable information and allows the estimation of their bioavailability (Elless et al., 2000). Stimulated gastic juice containing a solution of pepsin, sodium chloride and hydrochloric acid was used by Barmudez-soto et al. (2007) to release the metals from food and dietary supplements.

Plantain had been reported to contain a lot of iron (Fe), potassium (K) and other essential minerals (Baiyeri, 2000; Adeniji et al., 2007). This study was therefore aimed at determining the mineral content of plantain based products, and to ascertain the bioavailability of these minerals using the in-vitro digestion method. This will provide information of the percentage soluble fraction, available for absorption.

2. Materials and Methods

Plantain cultivar (agbagba) was collected from the International Institute for Tropical Agriculture (IITA), high rainfall station, Onne near Port Harcourt, Nigeria. Bambara groundnut (*Vigna subterrernea L verde*) seeds were purchased from markets in Enugu, Enugu State, South East, Nigeria.

2.1 Preparation of Plantain and Bambara Groundnut Flours

The methods of Adeniji et al. (2007) and Barimalaa et al. (1994) were used in the production of plantain flour and bambara groundnut flours, respectively.

2.2 Preparation of Bambara Groundnut Protein Concentrate

The protein concentrates from bambara groundnut flour was prepared using the alkaline wet extraction process described by Giami and Isechei (1999), for fluted pumpkin seed and adopted for bambara groundnut protein concentrate as earlier reported by Kiin-kabari and Giami (2015).

2.3 Products Formulation

The plantain based products include "amala" bread, cookies and cakes from composite flours of plantain and wheat enriched with bambara groundnut protein concentrate.

2.3.1 Amala

"Amala" is a common plantain paste, also called "elubo" by the Yoruba tribes in Western Nigeria was produced by stirring plantain flour with 15% bambara groundnut protein concentrate in hot water (1:4) until smooth paste was formed. A control sample of 100% plantain flour was also prepared.

2.3.2 Bread

The batter method described by Ogazi (1984) was used with modifications. The flours and ingredients were weighed; the yeast and a portion of the sugar were dissolved in warm water at 30 °C. The remaining sugar was dissolved separately. The flour, fat and salt were mixed together for about 5 min. The dissolved yeast – sugar solution was added and the mixing continued for 30 min. The resultant batter was scaled (500 g), proofed for 15-20 min and baked at 100 °C for 1 h.

70% wheat flour, 20% plantain flour and 10% bambara groundnut protein concentrate was used in bread and cakes while for amala and cookies, 85% plantain flour, 15% bambara groundnut protein concentrate were used as recommended by Kiin-Kabari et al. (2015).

2.3.3 Cookies

The cookies were produced from 85% plantain flour and 15% bambara groundnut protein concentrated as earlier reported by Kiin-kabari and Giami (2015). The procedure described by Arisa et al. (2013) was used, sugar (75 g) was added to 125 g of margarine in a Kenwood mixer and mixed at medium speed until fluffy. Egg (whole egg) and milk (powdered milk) were added while mixing and then mixing continued for 30 min. Sifted plantain flour (85%) and bambara groundnut protein concentrate (15%), baking powder and flavour were slowly added to the mixture. The mixture was kneaded to form a dough. It was then rolled on a flat board sprinkled with flour to a uniform thickness of about 0.4 cm. Circular cookies of 5.8-6.0 cm diameter were cut, placed in oiled baking trays and baked at 160 °C for 15 min.

2.3.4 Cake

Cake was produced using the creaming method of blending. Half (½) of the composite flour blends (70% wheat flour, 20% plantain flour and 10% bambara groundnut protein concentrate) were mixed with all the fat for about

2 min to obtain a creamy dough before adding the remaining composite flour and other ingredients like eggs, baking powder and water. More water was added gradually and mixing continued until the dough was soft and greasy. The dough was moulded into rolls, shaped and baked in the oven at 200 °C for 10 min.

2.4 Mineral

Mineral analysis was done by dry ashing according to procedure 14.013 of AOAC (2012). Muffle furnace (Model SKL, China) at temperature of 550 °C was used for ashing. After sample preparation, total mineral determination was done using Atomic Absorption spectrophotometer (AAS) (Hitachi Z-5300, polarized Zeaman, Hitachi Ltd, Japan). The light source was Hollow cathode lamp of each element, using acetylene and air combinations, with air pressure of 0.3Mpa, and air flow rate of 6.5L/min, acetylene pressure of 0.09Mpa and a flow rate of 1.7 L/min was used. Other operating conditions such as wavelength and lamp current are given for each element as follows: Ca = 422.7 nm and 2 mA, Fe = 248.3 nm and 2 mA, K = 766.5 nm and 1 mA, mg = 285.2 nm and 1mA, Na = 589.0 nm and 1mA.

Phosphorous was determined by molybdenum blue method and the absorbance read at 700nm using a spectrophotometer uv-visible (CELiL model CE2021 U.K).

2.4.1 Mineral Bioavailability Using *In-Vitro* Enzyme Digestion

Plantain product – ‘amala’, bread, cakes and cookies were subjected to in-vitro enzymatic digestion with pepsin plus pancreatin according to the method described by Ikeda (1990). Enzyme solution containing 16 mg pepsin (cat No. P6887) and 3.5 ml of 0.06 N Hcl, 1.0 g sodium chloride made up to 100 ml with deionised water was prepared. Another solution containing 1.6 g of pancreatin, (cat No. P1750) dissolved in phosphate buffer (pH 7.5) and made up to 100 ml with same buffer was also prepared. In a test tube, 20 ml of pepsin enzyme solution was added to 0.5 g of the sample.

The closed test-tube was shakened and incubated at 37 °C for 3 h. Immediately after peptic digestion, pH was adjusted to 8.0 using phosphate buffer. Toluene was added to the buffer to prevent the growth of micro organisms. Pancreatin solution (25 ml) with deoxycholate (1.0%) was then added to the digestion mixtures and samples were subsequently incubated for 20 h at 37 °C. After digestion, the suspensions were placed in ice-cold vessel and then clarified by centrifugations at 10,000 rpm for 20 min. The supernatants obtained were subjected to mineral analysis using atomic absorption spectrophotometer (AAS). The results obtained were used in calculating the percentage of minerals in the plantain products that are bioavailable in soluble fractions using the formula;

$$\text{Mineral in soluble fraction (\%)} = \frac{x}{y} x \frac{100}{1}$$

Where x = Minerals in soluble forms after digestion

y = Total minerals

2.5 Statistical Analysis

The data obtained were analysed using analysis of variance (ANOVA) with SPSS 16.0 software version 2007.

3. Results and Discussion

The calcium content of the enriched plantain products (‘amala’, bread, cake and cookies) ranged from 217 µg/g (amala) to 500 µg/g (cake) as shown in Table 1.Cake was observed to contain the highest level of total calcium compared to other products. This difference may be attributed to the ingredients such as eggs used in the product formulation. Although, the values obtained in this study are higher than 66/µg/g ca, reported by Izonfou and Omauru(1988) for green plantain, it is consistent with the work of Adeniji and Tenkuano, (2008) who reported 200 µg/g.ca for “Agbagba” cultivar.

The Food and Nutrition Board of the National Research Council (NRC/NAS, 1974) reported that an adult male and female require 350 mg and 300 mg of magnesium (mg) daily, respectively. The magnesium content ranged from 700 µg/g (amala) to 1,002 µg/g (cake). This suggests that between 428.6 g and 500 g of amala can provide at least 100% of the daily requirement of magnesium for these categories of people. However, only 30 g and 35 g of cake will be required to meet the daily magnesium needs of these people.

The level of potassium (k) in the enriched products ranged from 7,550 µg/g as observed in plantain cookies to 10,215 µg/g in composite bread prepared from wheat/plantain/BGPC blends. Although the highest value of potassium was recorded in bread, it was not significantly different with the value obtained in plantain cake (9,500 µg/g), as shown in Table 1.

These values are higher compared to 8,400 µg/g reported by Izonfou and Omauru, (1988) and 6,500 µg/g recorded by Baiyeri (2000) in plantain pulp at the unripe stage. This observation clearly suggests that bambara groundnut protein concentrates not only improved the protein content of the products investigated but also the mineral content, with the highest increase in potassium recorded in cookies (9.8%).

Table 1. Mineral composition of plantain products enriched with bambara groundnut protein concentrates (µg/g)

Products	Ca	Mg	K	Fe	P	Na
Amala (control)	194^{d}	592^{d}	8520^{c}	$5,012^{c}$	962^{b}	42.8^{b}
Amala*	217^{d}	700^{c}	$9,100^{b}$	$5,412^{c}$	1000^{b}	44.2^{b}
Bread (control)	391^{b}	778^{b}	9728^{a}	$5,747^{b}$	1184^{a}	46.2^{a}
Bread+	412^{b}	816^{b}	$10,215^{a}$	$6,015^{a}$	1216^{a}	48.6^{a}
Cake (control)	458^{a}	927^{a}	9258^{b}	$6,815^{a}$	1169^{a}	48.8^{a}
Cake +	500^{a}	$1,002^{a}$	9500^{a}	7100^{a}	1215^{a}	51.7^{a}
Cookies(control)	261^{c}	714^{c}	6814^{d}	$3,872^{d}$	855^{d}	41.1^{b}
Cookies*	315^{c}	800^{b}	$7,550^{c}$	$4,012^{d}$	908^{c}	42.3^{b}

a,b,c,d means bearing the same superscript within the same column do not differ significantly ($p > 0.05$).

* 85% plantain flour, 15% Bambara groundnut protein concentrate.

+ 70% wheat flour, 20% plantain flour and 10% Bambara groundnut protein concentrate.

The iron content of cake was higher (7100 µg/g) and significantly different from the other enriched plantain product studied, followed by bread (6015 µg/g), 'amala' (5412 µg/g) and finally the lowest was recorded in plantain cookies (4012 µg/g), with the highest increase of 7.4% observed in "Amala" and 6.0% in cookies when compared with the control. These values were higher than those reported by Ibrahium and Hegazy (2009) for whole wheat biscuits (465 µg/g) and 165 µg/g Fe reported by Leśniewicz et al. (2012), for breakfast cereals (cornflakes, Nestle brand). Also Giami et al. (2003) had earlier reported 130mg/g for wheat bread and 169 mg/g for bread supplemented with 10% fluted pumpkin seed flour. This observation confirmed the fact that plantain is higher in iron and its incorporation in baked product may be useful in fighting the widespread iron deficiency which is a nutritional disorder of public health significant in the developing countries (ACC/WHO, 1999; SCN 2000).

Phosphorous content of the enriched plantain product ('amala', bread, cake and cookies) were found to be within the range of 908µg/g and 1315µg/g. Although plantain bread and cake with values of 1216 µg/g and 1315µg/g, respectively, were not significantly different ($P > 0.05$). These values obtained were significantly different when compared to "amala" and cookies with 1000 µg/g and 908 µg/g respectively. This observation is similar to the report of Adeniji et al. (2007) who concluded that "Agbagba" a local plantain cultivar contain 1000 µg/g of iron and higher than 505.21 µg/g earlier reported in plantain (Baiyeri, 2000).

Sodium in the various plantain products developed was generally low. This observation further confirmed that plantain is a low sodium food. , The highest value for sodium (Na) was recorded in cake (51.7µg/g) and lowest value was observed in cookies (43.3µg/g). These values are lower when compared to 90.19 µg/g "Agbagba" reported by Adeniji et al. (2007) and 340 µg/g reported by Izonfou and Omauru (1988) for unripe plantain. Therefore, unripe plantain can be useful in formulating low sodium food products.

3.1 Bioavailability of Minerals

The release of six essential minerals as soluble forms after the in-vitro enzymatic digestion of the plantain products enriched with bambara groundnut protein concentrate in the preparation of bread, cakes, 'amala' and cookies are shown in Table 2. The study revealed that not all the minerals are bioaccesible; Mineral solubility has been widely employed in literature to predict mineral availability (Wolters et al., 1993; Hemalatha et al., 2007).

The percentage bioavailable calcium ranged from 26.3% (cookies) to 43.0% (cakes). However, there was no significant difference between soluble fractions of calcium in bread (40.9% and cake 43.0%). The least soluble fractions of 26.3% was found in "cookies" which was also significantly different ($p > 0.05$) when compared to"amala"(37.4%). These observations suggest that product formulation and processing methods such as fermentation improved bioavailability of calcium in the products studied. This is in agreement with the report of various researchers, who had earlier observed that fermentation is an efficient method of reducing the effect of phytic acid (PA) which binds metals such as ca, zn and fe, and therefore increasing the bioavailability of such minerals (Chaoui et al., 2003; Eltayeb et al., 2007). Similar observations had also been reported during the fermentation of "Tempe" by Mihiri et al. (2012). However, the Ca/P ratio are 1.1 and 0.8 for cakes and bread, respectively; and it would serve as good sources of calcium and phosphorous which are considered essential for bone formulation and teeth development in children. This observation was in agreement with the report of Ijarotimi et al. (2012) that statedthat food products containing a Ca/P ratio of > 1.0 is rated well while less than < 0.5 is rated poor.

The proportion of soluble fractions of magnesium (mg) released after the enzymatic digestion of plantain products with pepsin and pancreatin showed a range from 21.6% (cakes) to 28.7% (amala), while phosphorous (p) was between 19.2% (cakes) and 18.9% (cookies). These values for magnesium are close to 30% extraction efficiency of magnesium (mg) when pepsin was used to digest ready-to eat breakfast cereals. Lower than 50% extraction efficiency was reported for phosphorous by Lesniewicz et al. (2012).

Table 2. Soluble fractions (minerals) after in-vitro digestion of plantain products with pepsin and pancreatin (μg/g)

Minerals	Calcium(Ca)		Magnesium (mg)		Potassium (k)		Iron (Fe)		Phosphorous (P)		Sodium (Na)		
	Soluble Ca	% Soluble Ca	Soluble mg	% Soluble mg	Soluble k	% Soluble k	Soluble Fe	%Soluble Fe	% Soluble P	% Soluble p	Soluble Na	% Soluble Na	Ca/P
Amala	81.1^{c}	37.4^{b}	201.0^{b}	28.7^{a}	6200.0^{b}	68.1^{a}	3247.0^{b}	60.0^{a}	185.0^{b}	18.5^{a}	18.6^{b}	42.1^{b}	0.4^{b}
Bread	168.5^{b}	40.9^{a}	198.0^{b}	24.3^{ab}	6100.0^{c}	59.7^{b}	3188.0c	53.0^{b}	196.0^{a}	16.1^{b}	17.8^{b}	36.6^{c}	0.8^{a}
Cakes	215.0^{a}	43.0^{a}	216.0^{a}	21.6^{b}	6308.0^{a}	66.4^{a}	3564.0^{a}	50.2^{b}	200.0^{a}	19.2^{a}	21.4^{a}	41.4^{b}	1.1^{a}
Cookies	82.7^{c}	26.3^{c}	208.0^{b}	26.0^{a}	6182.0^{b}	61.8^{b}	2598.0^{d}	64.8^{a}	172.0^{c}	18.9^{a}	19.4^{a}	45.9^{a}	0.5^{b}

a,b,c, d means bearing the same superscript within the same column do not differ significantly ($p \leq 0.05$).

* 85% plantain flour and 15% bambara groundnut protein concentrate.

+ 70% wheat flour, 20% plantain flour and 10% wheat flour, 20% plantain flour and 10% bambara groundnut protein concentrate.

Although, plantain had been reported to contain high amount of iron (Fe) and potassium (k) by so many workers (Ogazi, 1984; Izonfouand Omauru, 1988; Baiyeri 2000; Adeniji et al., 2007), the proportions in soluble forms after enzymatic digestion of plantain products studied, suggests that not all the minerals are bioavailable, and absorbed by the intestinal cells. In this study, 59.7% (Bread) to 68.1% (amala) of potassium were solubilised. This observation is in agreement with the report of Ikeda et al. (2001) who reported a range of 60.1% K to 67.8% K released as soluble form after the Pepsin plus pancreatin digestion of flours of various bulk wheat varieties. It was also observed that iron (Fe) in cookies and 'amala' were 64.8% Fe and 60.0% Fe released in soluble forms and was significantly higher than what was released for bread (53.0% Fe) and cake (50.2% Fe). These reductions in bread and cakes may not necessarily be attributable to phytic acid content or other components of bakery products such as dietary fibre which can reduce iron bioavailability as reported by Swain et al. (2003), but calcium from milk especially in cakes has also been implicated to play an inhibitory effect on Iron absorption (Perales et al., 2006), decreasing its availability. Erdman (1981) studied rats fed with soy protein isolates and found iron bioavailability of 61% which is similar to what was recorded in plantain cookies (64.8%) and "amala' (60.0%), respectively. Although; the sodium (Na) content of the plantain products were low, the proportion of sodium in soluble forms ranged from 36.6% (bread) to 45.9% (cookies), which indicates that between 36.6%-45.9% of Na in the products studied are bioaccessible.

4. Conclusion

The plantain based products developed (bread, cakes, cookies and "amala") were high in potassium, iron and phosphorous and low in sodium. However not all the minerals detected in the products were bioavailable when digested enzymatically into soluble forms. Bread (59.7%) and amala (68.1%) of potassium were released into soluble form while 64.8% (cookies) and 60.0% (amala) of iron were bioavailable. The ca/p ratio for cakes and bread is high and can serve as good source of calcium and phosphorous which are considered essential for bone formation and maintenance in children and the aged.

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