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Effect of Iron-fortified 'Gari', Cassava Meal on Serum Iron, Hemoglobin Concentration and Total Iron-binding Capacity in Albino Wistar Rats

C.A. Emmanuel-Ikpeme, O. Igile Godwin and B.I.A. Mgbeje
Department of Biochemistry, University of Calabar, Calabar, Nigeria

Corresponding Author: C.A. Emmanuel-Ikpeme, Department of Biochemistry, University of Calabar, Calabar, Nigeria

ABSTRACT

The effect of iron fortified 'gari', a cassava fermented meal on serum iron, hemoglobin concentration and total iron binding capacity on Albino Wistar rats were investigated. The prevalence of Iron Deficiency Anemia (IDA) among the low income house-hold is high in developing countries due to low intake of dietary iron. Developing an intervention strategy for IDA is a major concern and food fortification is considered the sustainable strategy to alleviate IDA. Freshly grated cassava tubers were divided into four subsamples; A-C and then mixed with Ferrous Sulphate (FS), Iron (III) sulphate (F3) and Ferric Alum (FA), respectively in ratio 1:5000 (0.2 g fortificant kg^{-1} mash). The samples along with the Control (D) were each fermented in a solid state for 24 h and stir-fried to obtain gari granules. The gari samples were fed to rats divided into four groups of five for fourteen days and the serum, then analyzed for serum iron concentration, Total Iron Binding Capacity (TIBC) and hemoglobin concentration. The results showed significant increase ($p<0.01$) in serum iron and hemoglobin concentration and a decrease in Total Iron Binding Capacity (TIBC) levels in the rats fed with the fortified gari samples (A-C), compared with the control (unfortified). Rats in group A had significantly higher level of serum iron (21.73±1.3 μmol L^{-1}) and hemoglobin (13.63±2.8 g dL^{-1}) and significantly lower level (55.5±2.2 μmol L^{-1}) of TIBC($p<0.05$), indicating a better iron bioavailabilty. The findings suggests that fortification of gari with Ferrous Sulphate (FS) had higher bioavailability of iron and therefore hold promise in combating iron deficiency anemia.

Key words: Fortification, iron deficiency anemia, hemoglobin, iron-fortified, cassava meal, gari, total iron-binding capacity

INTRODUCTION

The human body requires iron as an essential component of life physiology and its deficiency (Sideorpenia or hypoferremia) is considered as the number one nutritional disorders in the world (WHO/NHD/01.3, 2001; CDCP, 1998). Iron is present in all cells in the human body as heme proteins and is needed in the body for many functions. It is a key ingredient in the synthesis of the oxygen carrying proteins; hemoglobin (which carries oxygen from the lungs to the tissues) and myoglobin (stores and uses oxygen in muscles). It also serves as a prosthetic group of enzymes that catalyse oxidation reactions and cytochromes, an important component of the electron transport chain. Very low level of iron in the body can affect these essential functions, which might result in morbidity and death. When the intake of iron does not meet the dietary daily iron needs, a negative iron balance develops, which then results in the depletion of the stored form of iron, while the iron status marker; blood hemoglobin is unchanged.

The advanced stage of iron depletion is Iron Deficiency Anemia (IDA), which results when the stored iron are deficient and blood levels of iron cannot meet daily needs. Iron deficiency anemia results in low levels of blood hemoglobin (Institute of Medicine, 2001). There is increased risk for small early (preterm) babies during pregnancy as a result of iron deficiency (Brownlie *et al*., 2004; Haas and Brownlie, 2001) as well as delay normal infant motor function or mental function (Friel *et al*., 2003; Lozoff *et al*., 2003; Grantham-McGregor and Ani, 2001). Memory or other mental functions could be affected in teens, thereby causing fatigue that could impair the ability to do physical work in adults (Bruner *et al*., 1996; Haas and Brownlie, 2001; Scholl *et al*., 1992). IDA could also cause a reduction in the immune function, hence increasing the susceptibility to diseases (Center for Disease Control and Prevention, 2002).

In IDA patients, the blood examination would show microcytic anemia, low serum ferritin, low serum iron and high Total Iron Binding Capacity (TIBC) (Center for Disease Control and Prevention, 2002). TIBC measures how much of the transferrin in the blood is not carrying iron. High level of transfer in that has no iron indicates IDA. The blood iron is transported in the cells tightly bound with iron protein transferrin. Groups that are most prone to developing this disease are young children, pre-menopausal women (due to menstruation) especially pregnant women (because of higher iron need) and people with restrictive diet (e.g., vegetarians) (Stoltzfus *et al*., 2004). Iron is not synthesized by mammalian tissues and is obtained from dietary sources. Inadequate dietary iron can thus result in iron deficiency. Eating a healthy diet that includes good source of iron and food fortification with iron is generally considered the best strategy to prevent and combat iron deficiency (Watanapaisantrakul *et al*., 2007). Healthy diets that are good sources of iron include fruits, vegetables, whole grains, milk products, meat, fish, eggs, beans and a good source of vitamin C to promote uptake (CDCP, 1998). In Africa and indeed in many developing nations, most of these iron-rich diets (especially the heme iron sources) and iron supplements are beyond the reach of low income families. It is not surprising that IDA affects mostly people of low socio-economic status (Foege, 2002). Indeed, it has been reported that the prevalence of iron deficiency is high in developing countries mainly due to the low intake of bioavailable iron (Yip and Ramakrishnan, 2002). A sustainable intervention strategy to address iron deficiency will be to fortify an affordable staple food with iron.

Gari is a common household staple food in the West African sub region made by fermenting cassava tubers (*Manihot utilisima*). The cassava tuber consist of 80% carbohydrates (Vlavonou, 1989) making it a high source of energy but low in trace mineral (Adom *et al*., 2010). Food fortification does not require compliance, as it can be introduced into existing food system and hence can play a major role to improve diet quality, with respect to the micronutrient need of the population. Hence, gari is considered a good vehicle for iron fortification. Ikpeme-Emmanuel *et al*., (2011) reported a significantly ($p<0.05$) higher iron values of 10.70±0.30, 8.80±0.10 and 12.40±0.10 mg 100 g^{-1} for gari fortified with ferrous sulphate, Iron III sulphate and ferric alum, respectively compared with an iron value of 1.01±0.10 mg 100 g^{-1}. However, few study (Ebuehi and Mbara, 2011) have focused on the bioavailability of iron from iron fortified gari. Hence, the aim of this study was to evaluate the iron bioavailability of iron salts used for gari fortification.

MATERIALS AND METHODS

Materials: Fresh mature cassava was bought from a farmer in Akamkpa Local Government Area of Cross River State, Nigeria. Ferrous Sulphate, Iron III Sulphate and Ferric Alum chemicals and

kits for experimental assay were purchased from Sigma Aldrich (Germany). Albino Wistar rats were obtained from Animal Science Department, Faculty of Agriculture, University of Calabar, Nigeria.

Gari preparation and fortification: Cassava tubers were flushed with tap water and then washed in distilled water before grating with an electrically powered grater into fine mesh. The mesh was divided into four Batches A, B, C and D. Batches A, B and C were fortified with ferrous sulphate (FS), iron III sulphate (F3S) and Ferric Alum (FA), respectively in the ratio 1:5000 (i.e., 0.2 g fortificant to 1 kg gari). Batch (D) was the control batch and was not fortified. The four samples were placed in sterile separate cotton bags and fermented in a solid state for 24 h and then dewatered. The dewatered mash was removed from each bag, sieved and the fibrous parts discarded. Gari was made by stir-frying the fine wet mash, free from fibrous materials, in a steel pan over an open fire until dry to obtain granules.

Experimental design: Completely randomized design was used for the study. All animal experiments in this study were conducted in compliance with the guidelines of the Nigerian Assoc. Laboratory Animal Science. Ten weeks old twenty albino Wistar rats weighing between 80-150 g of either sex was used for the study in duplicates. The rats were divided into 4 groups of five each and housed at room temperature (25°C) in cages with a 12 h light and dark cycle. The first 3 groups were fed with gari made from sample A (FS), B (F3S) and C (FA) respectively, while group D were fed with the unfortified D gari sample. Water was given *ad libitum*. The animals were fasted for a period of 12 h prior to treatment and allowed access to only water, after which they were fed for 14 days with fortified or unfortified gari diets. A period of 3 days was allowed for acclimatization, before biochemical analysis was done on relevant tissues.

Analysis: After 14 days of feeding, the body weights were again determined and fasting blood extracted for analysis. The blood sample collected were transferred into labeled centrifuge (S3000) tube and spun at 1000 rpm for 5 min in a centrifuge to separate serum from red blood cells. A sterile pasture pipette was used to transfer the serum supernatant, from the pelleted red blood cell into labeled serum containers and stored in the refrigerator at 4°C until needed.

Estimation of serum iron: Serum iron concentration was determined according to a method developed by International Committee for Standardization in Hematology, ICSH (1978). Serum (0.5 mL) was added to 0.5 mL of iron standard, 0.5 mL of iron free water and 0.5 protein precipitant. The mixture was mixed vigorously, allowed to stand for 5 min and then centrifuged at 1300 g for 4 min. The supernatant was collected, treated with a chromagen solution and the absorbance measured at a wavelength of 562 nm using a spectrophotometer (SL300). The concentration of serum iron was calculated from the equation.

$$\text{Serum iron} = \frac{\text{Absorbance of test-Absorbance of blank solution}}{\text{Absorbance of iron standard solution-Absorbance of blank solution}} \times \text{Concentration of standard}$$

Estimation of total iron-binding capacity (TIBC): Total iron-binding Capacity (TIBC) was determined using colorimetric methods (ICSH, 1978). Serum (0.5 mL) was placed in 1.5 mL eppendorf tube containing 0.5 mL saturated iron solution and mixed vigorously. Light magnesium carbonate (100 mg) was then added to the mixture and mixed vigorously and then allowed to stand

for 30 min before centrifuging at 1300 g for 4 min. The supernatant (0.5 mL) was collected and the absorbance measured at 650 nm using a spectrophotometer (SL300). TIBC was obtained by multiplying the result by 2.

Estimation of hemoglobin concentration: The cyanomethemoglobin method of the ICSH (1987) was used. Blood sample (20 μL) was diluted with 4 mL of diluents containing potassium cyanide and potassium ferricyanide and allowed to stand for 5 min. Absorbance was measured at 540 nm using a spectrophotometer (SL300) against a blank. The result was compared with a commercially produced standard. Hemoglobin concentration was then calculated from the Eq.:

$$\text{Hb (g L}^{-1}) = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times (\text{Standard}) \times \frac{\text{Dilution factor}}{1000}$$

Data analysis: All experiments were repeated in 3 sets independently and each having 3 replicates. The means and Standard Deviations (SD) were calculated taking all the readings in consideration. SPSS (Statistical Package for Social Sciences) was used to analyze for student t-test, Pearson correlation and ANOVA (significance $p \leq 0.01$).

RESULTS

Effect of fortification on serum concentration: The serum concentration profile of the four groups of rats based on the type of feed given is shown in Table 1. Serum iron measures the amount of circulating iron that is bound to transferring. The results showed significant increase ($p<0.01$) in serum iron concentration in the groups fed with fortified gari compared with the control group fed with unfortified gari. The mean serum iron values ranged from 9.7±2.1 μmol L^{-1} for Control to 21.7±1.3 μmol L^{-1} for group A. The serum iron concentration for rats in group A were significantly ($p<0.01$) higher than serum iron concentration of other fortified groups. Rats fed the Control group had the lowest serum iron concentration which was considered anemic (9.7±2.1 μmol L^{-1}). Although the mean value of group C was not anemic (14.8±2.2 μmol L^{-1}), two members of the group fell under the base line of anemic group.

Effect of fortification on total iron-binding capacity (TIBC): Table 2 shows the total iron-binding capacity (TIBC) of rat groups fed with gari samples. TIBC indicates the total amount of iron needed to saturate plasma or serum transferrin, a binding protein with iron (Brittenham *et al.*, 1981). The TIBC values ranged from 55.5±2.2 μmol L^{-1} (Group A) to

Table 1: Serum iron concentration of rats fed with fortified and unfortified gari (μmol L^{-1})

No. of rats	Control	Group A	Group B	Group C
1	7.4	25.5	21.1	15.0
2	9.2	18.1	17.9	14.9
3	8.7	21.0	16.8	14.3
4	10.3	19.9	23.1	18.3
5	13.1	24.0	20.1	11.5
Mean±SD	9.7±2.1	21.7±.1.3*	19.6±1.6*	14.8±2.2*

*Significant difference from control group ($p<0.01$), Group A: Ferrous sulphate fortificant, Group B: Iron lll sulphate fortificant, Group C: Ferric alum fortificant, Control: Unfortified

Table 2: Total iron-binding capacity of rats fed with fortified and unfortified gari (μmol L^{-1})

No. of rats	Control	Group A	Group B	Group C
1	80.1	63.2	68.1	63.9
2	77.5	48.5	59.7	60.2
3	79.3	55.5	64.2	70.1
4	70.4	50.0	60.1	59.3
5	64.8	60.1	65.1	76.2
Mean±SD	74.4±2.5	55.5±2.2*	63.4±1.5*	67.7±2.1*

*Significant difference from control group ($p<0.01$). Group A: Iron ll sulphate fortificant, Group B: Iron lll sulphate fortificant, Group Cl: Ferric alum Fortificant, Control: Not fortified

Table 3: Hemoglobin concentration of rats fed with fortified and unfortified gari (g dL^{-1})

No. of rats	Control	Group A	Group B	Group C
1	9.85	14.32	13.73	11.97
2	9.80	12.96	12.94	11.76
3	9.75	13.71	12.11	11.59
4	9.86	13.14	14.02	13.11
5	9.50	14.02	13.09	11.28
Mean±SD	9.75±1.5	13.63±2.8*	13.18±3.4*	11.94±3.0*

*Significant difference from control group ($p<0.01$). Group A: Iron ll sulphate fortificant, Group B: Iron lll sulphate fortificant, Group C: Ferric alum Fortificant, Control: Unfortified

74.4±2.5 μmol L^{-1} (Control). The TIBC values of the rats in groups A, B and C were lower than those in the Control group ($p<0.01$), the values increased progressively from group A to Control.

Effect of fortification on hemoglobin concentration: Hemoglobin values of rats fed with fortified and unfortified gari is shown in Table 3. Serum hemoglobin measures the level of free hemoglobin in the liquid part of the blood (the serum). Free hemoglobin is the hemoglobin outside of the red blood cells. The hemoglobin values ranged from 9.75±1.5 g dL^{-1} for control group to 13.63±2.8 g dL^{-1} for group A. There was a significant difference ($p<0.01$) in the concentration of hemoglobin between the rats fed with fortified gari (Groups A-C) and the rats fed with unfortified control sample . The highest hemoglobin level ($p<0.05$) recorded was for group A rats (13.63±2.8 g dL^{-1}), while the least value ($p<0.05$) was for rats in the control group (9.75±1.5 g dL^{-1}).

Correlation among serum iron, total iron binding capacity and hemoglobin: To explore interactions between the indicators of iron deficiency, a correlation analysis was done, as shown in Tables 4 and 5. There was a high and significantly positive correlation between serum iron and iron binding capacity (TIBC) levels of group A and group A (0.983) group A and B(0.947) and a high negative correlation of both controls of the indicators(-0.963). A high positive correlation were observed between hemoglobin levels with serum iron levels in both group As(0.973), group B and group B(0.952), group C and group B(0.724), group C and group C(0.957), and also a positive correlation was observed between the controls (0.728).

Table 5 showed a high positive correlation coefficient between TIBC and hemoglobin with group A and group A(0.997), well as between group B and group A(0.990) . A high negative correlation was observed for group C and group C (-0.752), as well as between the controls (-0.849).

Table 4: Correlation coefficient of serum iron with total iron binding capacity and hemoglobin

	Serum Iron			
	Control	Group A	Group B	Group C
TIBC				
Group A	-0.034	0.983*	0.103	-0.545
Group B	-0.196	0.947*	0.100	-0.498
Group C	0.559	0.529	-0.321	-0.881*
Control	-0.963*	0.098	-0.441	0.239
Hemoglobin				
Group A	-0.061	0.973*	0.074	-0.571
Group B	0.019	0.221	0.952*	0.571
Group C	-0.195	-0.310	0.724*	0.957*

*Significant ($p<0.01$) from control group. Group A: Iron ll sulphate fortificant, Group B: Iron lll sulphate fortificant, Group C: Ferric alum fortificant, Control: Unfortified, TIBC: Total iron binding capacity

Table 5: Correlation coefficient of total iron binding capacity and hemoglobin

	TIBC			
	Control	Group A	Group B	Group C
Hemoglobin				
Group A	0.031	0.997*	0.990*	0.610*
Group B	-0.272	0.047	-0.029	-0.503
Group C	0.024	-0.451	-0.449	-0.752*
Control	-0.849*	-0.335	-0.473	-0.030

*Significant ($p<0.01$). Group A: Iron ll sulphate fortificant, Group B: Iron lll sulphate fortificant, Group C: Ferric alum fortificant, Control: Unfortified, TIBC: Total iron binding capacity

DISCUSSION

The rats fed with iron-fortified gari showed a significant increase in serum iron and hemoglobin concentration in relation to the rats fed with unfortified gari ($p<0.01$). This significant increase in hemoglobin and serum iron concentration is indicative of the increased bioavailability of iron as a result of the iron forticants present in the gari. Rats fed with ferrous sulphate fortified gari (Group A) had the highest concentration of serum iron, indicating that the iron from the salt was better absorbed in the blood. This might be due to the interaction between the salt and the gari. The primary ingredient for hemoglobin synthesis is iron and its production is proportional to the concentration of iron in the serum. A recent study on the effect of iron fortified gari fed to phenyl hydrazine-induced anemic rats (Ebuehi and Mbara, 2011) also reported an increase in hemoglobin levels when anemic rats were fed with gari fortified with Ferazone ($Na_2FeEDTA$). The significantly higher ($p<0.05$) hemoglobin levels observed is also consistent with findings from other studies on fortified foods (Adom *et al*., 2010; Bradley *et al*., 1993; Walter *et al*.,1993; Lartey *et al*., 1999).

There was a significant decrease ($p<0.01$) in Total iron-binding capacity (TIBC) in rats fed with fortified gari compared with the control fed with unfortified gari. This is indicative of increased bioavailability of iron in rats fed with the fortified diet. Among the fortified samples, the mean TIBC value for group A was significantly ($p<0.05$) lower than for Groups B and C, which indicates better iron stores for rats in group A and consequently better bioavailability of iron by Ferrous sulphate. The TIBC values increased as need for iron increased and this was seen in the control group whose level was raised due to deficiency of iron in serum. The rats in group A experienced a much lowered

TIBC indicating an increased concentration of iron in ferrous sulphate fortified group. Groups B and C where both relatively similar and indicate the normal TIBC level for a healthy individual. Total iron binding capacity increases as the need for iron increases (Davidson *et al*., 1975). This is as a result of more transferrin being released to scavenge for iron.

The concentration of hemoglobin in blood reveals the state of an individual with respect to iron deficiency. Groups A and B showed a much increased level of hemoglobin while group C was slightly lower and some animals in that group actually fell below the base line of normal hemoglobin levels. However, group A fed rats had the highest ($p<0.05$) level of hemoglobin.

Generally, the result indicated that the iron salts actually had access into the system and where available for hemoglobin synthesis, however, Group A rats fortified with ferrous Sulphate indicated better hemoglobin synthesis.

Although, all three fortificants used in the study resulted in increase in the bioavailability of iron, as evidenced by the blood analysis, ferrous sulphate (Group A) proved to be a better fortificant followed by Iron (III) sulphate (Group B) and to a lesser extent Ferric alum (Group C). Though, Ikpeme-Emmanuel *et al*. (2011) reported that there was no difference ($p<0.05$) in the overall acceptability panelists ratings in the gari fortified with either Iron III Sulphate or ferric alum with the control. However, the iron bioavailability of Ferrous Sulphate showed a significant ($p<0.05$) better effect in the indicators, suggesting that Ferrous Sulphate hold potential for use in IDA intervention.

Food fortification is recognized as an important strategy for controlling micronutrient deficiency (Chen *et al*., 2005; Davidsson, 2003). According to the Food and Agriculture Organisation (FAO/WHO, 1994; Allen *et al*., 2006), a vehicle used to convey micronutrients must meet the following criteria: it must be commonly consumed by the target population; have a constant consumption pattern with a low risk of excess consumption, have good stability during storage; be relatively low in cost; be centrally processed with minimal stratification of the fortificant; have no interaction with the fortificant; be contained in most meals with the availability unrelated to socio-economic status and be linked to energy intake. Gari, the vehicle used in this study, meets all this criteria. The fortificants used also meet the selection criteria for fortificants i.e. bioavailability, cost and safety (Hurrel and Cook, 1990). Besides, the integration of the fortification process employed in this study with the normal processing of gari is within the socio-economic and socio-cultural framework of the people in the sub region where it is a staple food and is thus sustainable. Furthermore, the fortification was achieved with little interference with the normal food preparation technique. The only other study on fortified gari available in the literature (Ebuehi and Mbara, 2011) fortified the gari after processing by soaking the finished product in the fortificant and then drying before presentation. This would meet less acceptance to the human population compared with the fortification method employed in this study.

CONCLUSION

Iron status, as indicated by serum iron, hemoglobin concentration and Total Iron Binding Capacity (TIBC), was enhanced by gari fortification with ferrous sulphate and iron III sulphate, however a higher serum iron content was achieved with ferrous sulphate. The potential for nutritional exploitation of iron fortification of gari with ferrous sulphate is further enhanced by the fact that the technology can be easily be adopted by local gari processors. Consequently, long term sustainability of prevention of iron deficiency in Western Africa may depend on this strategy. This study is a pilot to extending the study to human population in the quest to prevent and reverse iron deficiency.

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