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Determining the effect of heat treatment on iron fortified soybean *gari* blend and its bioavailability

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ABSTRACT

Gari is a cassava based food product which lacks most essential nutrients needed to promote good health and growth. An easy-to-adopt strategy widely accepted and used by most low-income household to improve nutritional intake is to blend nutrient dense and nutrient-poor agricultureal produce in a meal. Soybean is used in food blends, as it contributes to caloric source especially supplementary protein. Micronutrient deficiency has been a major challenge in middle and low income countries. The most prominent of micronutrient deficiencies is iron deficiency, which has a potential harmful developmental effect especially on infants, adolescent girls, pregnant women and the elderly. *Gari* is widely consumed in Ghana and along the West African coast, therefore can be a good medium for food fortification to improve nutrition. The objective of the study was to fortify defatted soybean *gari* blend with iron and evaluate the effect of heat treatment on anti-nutrient content, estimated iron bioavailability and colour of the products. A known quantity of commercial food grade ferric sodium (FeNa) EDTA was added to cassava mash (with or without commercial food grade defatted soybean flour) before and after processing into *gari*. The elemental composition, anti-nutritional properties, estimated iron bioavaliability and colour of the product were determined using appropriate analytical methods. Results showed that fortification with iron improved the iron content of the *gari* samples (with or without defatted soybean flour). Addition of iron to defatted soybean gari blend before heat treatment significantly ($p<0.05$) decreased the lightness (L^*) and yellowness (b^*) of the end product. Blending *gari* with soybean flour elevated its anti-nutrient content; however the estimated molar ratios of anti-nutrients to iron and zinc contents were within acceptable levels as stipulated by USAID and FAO guidelines. Iron fortification of soybean *gari* blend has huge potential to reduce iron deficiency anaemia and protein-energy malnutrition among *gari* consumers in Africa.

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Introduction

The prevalence of micronutrient deficiency in sub-Saharan Africa is still soaring even though there have been great efforts by local and international organizations to reduce it. The rate of increase varies across different nutrients and levels of development [35]. Deficiency in micronutrient is detrimental to people of all ages, however, there is a limitation in achieving optimal cognitive and intellectual ability in children and adolescence whilst in pregnant women, it support normal foetus development and health [14,27]. Anaemia caused by iron deficiency is the most common micronutrient deficiency across the world and nutritional centred interventions have been the prime strategy to reduce it [6,39,40].

Food synergy has been an economic strategy to improve nutrition especially in developing countries [28]. Blending a variety/diverse of food is a viable way of increasing the nutrient density of a meal. The use of animal as a source of nutrition is very effective in reducing the prevalence of micronutrients deficiency, however, due to cost and some social economic challenges, smallholder livestock farmers in Ghana would prefer using their livestock as emergency funds than improving nutrition of their household [32]. Household dietary diversity is also greatly dependant on household income and nutritional awareness [1,5,13,25]. The use of plant based protein is very economical. Legumes especially soybean, have been used to improve household nutrition; however these legumes contain anti-nutrients which affects the bioavailability of certain nutrients. According to Bora [7], these anti-nutrients can cause detrimental effects to humans by impairing food intake, micronutrient uptake or utilization.

Another effective strategy to reduce micronutrient deficiency is food fortification. Addition of micronutrient to processed food has helped to reduce the prevalence of specific micronutrient amongst target group [12,29]. Research by Kruger, [26], has shown that iron fortification can be an effective strategy against nutritional iron deficiency. Fortification of whole maize flour with iron has been reported to improve the iron status of school children in Kenya [4]. Iron fortification is the most challenging of all food fortification processes as the iron form used may not be readily bio available to the target group. Ferric Sodium EDTA (FeNa EDTA) is the most bio available iron form, however this iron form cast sensory defects such as changes in colour, flavour and odour on foods, [29], after processing and during storage.

For any food fortification program to be successful the food vehicle selected for the fortification should be widely consumed by the target population and its production should be on a large scale all year [10]. *Gari is* a white to cream granular cassava (*Manihot esculenta* Crantz) product, widely consumed as household staple food along the West African coast. In Ghana, this economical household staple is largely consumed by adolescents in boarding schools and it is also included in the menu of the Ghana's school feeding program [2]. *Gari* is basically carbohydrate with minimal levels of other essential nutrients needed to support healthy human growth. Due to its low nutritive value, there have been research studies to improve its nutritional quality which include but not limited to bio-fortification of the cassava tubers, cassava mash blending and fortification. This research study froms part of a fodd product development research project to improve the nutritional quality of this economical household staple food without changing the organoleptic qualities of the final developed fodd product. In other research works reported in the area of gari fortification, iron or soybean flours are used separately. The present study however utilized defatted soybean flour and iron to fortify gari to enhance the nutritional quality of gari. In so doing, there is a need to investigate the influence of heat treatment on the added soybean flour and iron bioavailability. The present study therefore aimed at determining if two iron fortification stages during processing can affect product colour and iron bioavailability of fortified soybean *gari* blend.

Materials and methods

Materials

The materials used for the fortification of soybean *gari* blend were cassava, defatted soybean flour, and FeNa EDTA then high density polyethylene bags.

Source of materials

Cassava roots ('Bosom nsia' variety) were procured from farmers at Korkormu, near Koforidua in the Eastern Region of Ghana. Commercial food grade defatted soybean flour (≈2 % fat, hexane defatted)was donated by the American Soybean Association (ASA). Food grade FeNa EDTA (commercial) was obtained from SternVitamin GmbH & Co. KG. This ferric sodium FeNa EDTA was specifically engineered for this product development, hence the moisture content and fortification procedure for the gari were greatly considered.

Mixture model

Estimation of micronutrient quantity was based on recommendation from literature on micronutrient levels for fortification of various food blends [8,11]. The quantity of each material used in processing iron fortified soybean *gari* blend is represented in Table 1. Composition ratio of defatted soybean *gari* blend was 20 % defatted soybean flour to 80% dewatered cassava mash on dry matter bases.

Table 1
Quantity of materials used for iron fortified soybean *gari* blend.

Materials	Weight (g)*
FeNa EDTA	0.4
Defatted soybean flour	210.5
Cassava mash	1600

* *Calculation based on dry matter bases of the production of 1 kg iron fortified soybean gari blend end product.*

Methods

Preparation of unfortified raw gari

The cassava roots were sorted and cleaned, peeled, washed and milled into a mash. The mash were collected into woven polyethylene sacks, pressed to dewater for 24 h and allowed for spontaneous fermentation. The dewatered cassava mash with moisture content of 45-50 % was milled again to remove fibrous and large cassava pieces. Dewatered cassava mash was weighed and roasted at 70 ^{o}C average temperature for approximately 45 min (for every 6 kg of *gari* roasted) until it attained a final moisture content of 6 % and crispy. The roasted *gari* was allowed to cool (at room temperature, 27 ± 5 °C) and packaged in high density polyethylene bags and labelled raw unfortified *gari* sample A.

Preparation of iron fortified raw gari *(Before roasting)*

As mentioned above (2.2.1), the sieved dewatered cassava mash was weighed and 0.4 g of ferric sodium FeNa EDTA was added and mixed thoroughly. The fortified cassava mash was roasted at 70 ^{o}C average temperature for approximately 45 min (for every 6 kg of gari roasted) until it attained a final moisture content of 6 % and crispy. The roasted iron fortified raw *gari* was allowed to cool (at room temperature, 27 ± 5 °C), packaged in high density polyethylene bags and labelled iron fortified raw *gari* sample B.

Preparation of iron fortified raw gari *(After roasting)*

Dewatered cassava mash (2.2.1) was weighed and roasted at an average temperature of 70 ^{o}C for approximately 45 min (for every 6 kg of gari roasted) until it attained a final moisture content of 6 % and crispy. The roasted *gari* was allowed to cool (at room temperature, 27 ± 5 °C) and thoroughly mixed with 0.4 g of FeNa EDTA. The iron fortified raw *gari* was then packaged in high density polyethylene bags and labelled iron fortified raw *gari* sample C.

Preparation of unfortified soybean gari *blend*

The dewatered cassava mash mentioned above (2.2.1) was milled again, weighed and mixed with a known quantity of defatted soybean flour at the ratio of 80:20 (cassava mash: soybean flour) on dry matter basis. The mixed samples were roasted at 60 ^{o}C average temperature for approximately 45 min (for every 6 kg of gari roasted) until it attained a final moisture content of 6 % and crispy. The soybean *gari* blend sample was allowed to cool (at room temperature, 27 ± 5 °C), weighed, packaged in high density polyethylene bags and labelled soybean *gari* blend sample D.

Preparation of iron fortified soybean gari *blend (Before roasting)*

The dewatered cassava mash mentioned above (2.2.1) was sieved, weighed and mixed with the defatted soybean flour mixture at the ratio of 80:20 (cassava mash: soybean flour) on dry matter basis. A calculated quantity, 0.4 g of FeNa EDTA was weighed and mixed thoroughly with the blend of defatted soybean flour and cassava mash. The mixed sample was roasted at 60 ^{o}C average temperature for approximately 45 min (for every 6 kg of gari roasted) until it attained a final moisture content of 6 % and crispy. The soybean *gari* blend sample was allowed to cool (at room temperature, 27 ± 5 °C), weighed, packaged in high density polyethylene bags and labelled iron fortified soybean *gari* blend sample E.

Preparation of iron fortified soybean gari *blend (After roasting)*

The dewatered cassava mash mentioned above (2.2.1.) was sieved, weighed and mixed with a known quantity of defatted soybean flour at the ratio of 80: 20 (cassava mash: soybean flour) on dry matter basis. The mixed sample was roasted at an average temperature of 60 ^{o}C for approximately 45 min (for every 6 kg of gari roasted) until it attained a final moisture content of 6 % and crispy. The soybean *gari* blend sample was allowed to cool (at room temperature, 27 ± 5 °C) and thoroughly mixed with 0.4 g of FeNa EDTA. The iron fortified soybean *gari* blend was weighed, packaged in high density polyethylene bags and labelled as iron fortified soybean *gari* blend sample F.

Storage of samples

Packaged and labelled *gari* samples were kept in paper cartoons and stored at ambient temperature (27 ± 5 °C) until analysed.All Laboratory analyses were done within two weeks of sample preparation.

Anti-nutritional analyses

Anti-nutritional analyses were done using the method described by Haug & Lantzsch [19]; Price, [33] and Al-Wahsh, Yan & Liebman (2012). Phytic acid was extracted from approximately 2 g wet solid state *gari* samples by using 33 ml of 24 % HCl under continuous shaking (200 rpm) for one hour and determined according to the method by [19]. Tannin in gari was extracted using10-20 ml of 70 % methanol acidified with 1 % concentrated HCl. *Gari* samples were homogenized for 1 min at 10,000 rpm and subsequently centrifuged (4000× g) and supernatants collected in a clean flask. The extracts were evaporated using a rotary evaporator at 40 ^{o}C to dryness and the dry residues dissolved in 25 ml of absolute methanol prior to performing the vanillin- HCl assay. The condensed tannins were assayed colorimetrically by the method of Price, Scoyoc & Butler (1978).Oxalate contents were determined using the method of Al- Wahsh (2012). Gari samples were sieved to fine particles in a pan (125μm), 0.5 g was weighed into 250 ml volumetric flask and 50 ml of 2 N HCl for total oxalate extraction was added. The flask was placed in a shaking water bath at 80 °C for 30 min. The extract was further diluted with 50 ml of distilled deionized water and then transferred into 15 ml centrifuge tube and centrifuged at 4200 rpm for 10 min. The supernatants were filtered through Whatman #1 filter paper. The extracts were analyzed in triplicate for oxalate using a commercially available enzymatic kit (Trinity Biotech, Berkeley Heights, New Jersey), which is based on measuring the amount of hydrogen peroxide liberated from the oxidation of oxalate by oxalate oxidase.

Elemental analyses using the atomic absorption spectrophotometry (AAS)

The powdered sample was weighed (0.5 g) into a labelled 100 mL polytetrafluoroethylene Teflon bombs. 6 mL of conc. HNO_3 (65 %) and 1 mL of H_2O_2 (30 %) was added to the samples in a fume chamber. The samples were then loaded on a microwave carousel. The vessel caps were secured tightly. The complete assembly was microwave-irradiated for 20 min in a milestone microwave laboratory station (ETHOS 900 D model) using the following parameters; 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 5 min for 600 W with a pressure of 100 psi, and temperatures of 400 °C and 500 °C. Five minutes was allowed for venting [24]. After digestion, the Teflon bombs mounted on the microwave carousel were cooled in a water bath to reduce internal pressure and allow volatilized materials to resolubilize. The digest was made up to 20 mL with distilled water and assayed for the presence of iron, zinc, manganese, cadmium, magnesium, chromium, and lead in an acetylene air flame. Quality control and quality assurance were incorporated in the analytical scheme. Quality control was measures were checked to avoid contamination during analysis. The blank value procedure was used to determine the quantitative and qualitative determination limits of all the elements of interest. Reference standards for the elements of interest, blanks and repeats of the samples were digested the same way as the actual samples. These served as internal positive controls. The digested samples were then aspirated using Varian AA240FS fast sequential Atomic Absorption Spectrophotometer. The instrument was initially calibrated using 4 % acetone solution containing known amount of the elements of interest before the reading of any element with a standard solution of the element. A linearity of the calibration curve was always checked before the samples were aspirated. Calculation was obtained as stated below:

$$\text{Final concentration}(\text{mg/kg}) = \frac{\text{Concentration} \times \text{Normal volum}}{\text{Weight of sample in grams}}$$

Concentration recorded = given on the monitor attached to the instrument

Nominal volume = final volume after reagent and water were added. Weight of sample = 0.5 g

Estimation of molar ratio of phytate: minerals

Estimation of molar ratio of phytate to iron and zinc were calculation using the method as described by Gangchemg et al., [15]; Dahdouh et al., [9] as seen in equations 1 and 2 below.

Colour analyses

All *gari* samples were sieved to 3.35 mm particle size. The sieved *gari* samples were poured into a glass Petri dish (10 cm diameter, 2 cm depth) and scraped flat. A Hunter Lab colorimeter (aperture: 25 mm; Model 45/0, HunterLab Associates Laboratory Inc., Hong Kong, PRC) was used to determine colour values of the gari samples by first calibrating using the black and white tiles (X = 80.4, Y = 85.3, Z = 91.5) according to the manufactures instructions. In all five colour reading was taken for each gari sample at different places and reported as average L^*, a^*, b^* values.

Data analyses

Data was analysed using Microsoft Excel (2010) and statgraphics v. 16. Mean values and standared deviation were calculated from three replivates for elemental and anti-nutreint analyses, and five replications for colour analyses using Microsoft Excel (2010). One way ANOVA was performed to evalute significant difference at 5 % confidence level for all analyses using Stat graphics v. 1. except estimation of phytate to iron and zinc ratio. Sginificant means were separated using LSD at $p \leq 0.05$. Microsoft excel was used to calculate the phytate to iron and zinc ratio

Table 2
Anti-nutritional content of iron fortified soybean *gari* blends.

Sample ID	Anti-nutritive factors (mg/100g)		
	Phytates	Oxalate	Tannins
A	0.511 ± 0.003^{A}	0.048 ± 0.002^{C}	0.031 ± 0.001^{G}
B	0.510 ± 0.001^{A}	0.070 ± 0.002^{D}	0.027 ± 0.002^{E}
C	0.511 ± 0.003^{A}	0.063 ± 0.001^{F}	0.029 ± 0.003^{F}
D	1.022 ± 0.004^{B}	0.038 ± 0.002^{B}	0.008 ± 0.002^{A}
E	1.021 ± 0.003^{B}	0.039 ± 0.002^{A}	0.010 ± 0.001^{B}
F	1.021 ± 0.004^{B}	0.038 ± 0.002^{B}	0.013 ± 0.002^{C}
H	1.512 ± 0.006^{C}	0.199 ± 0.001^{E}	0.0140 ± 0.000^{D}

Values are means of triplicate experimants ± standard deviations of each sample with its mean separation as superscripts for each parameter. Means in the same column with the same superscripts are not significantly different ($p>0.05$) from each other.
Key
A= Raw gari unfortified; *B*= Iron fortified raw gari (before roasting); *C*= Iron fortified raw gari (after roasting).
D= Soybean gari (unfortified); *E*= Iron fortified soybean gari (before roasting); *F*=Iron fortified soybean gari (after roasting).
H= Soybean flour.

Results and discussion

Anti-nutritional content of iron fortified soybean gari *blend*

The results for the anti-nutritional contents of iron fortified soybean *gari* blend are presented in Table 2. Phytate, oxalate and tannins anti-nutrients were determined in the iron fortified soybean *gari* blend and defatted soybean flour.

The highest mean value for phytate was 1.512 mg/100 g for sample H (soybean flour) the lowest was 0.510 g/100 g recorded for sample B (ron fortified raw *gari,* before roasting). There were no significant difference in phytate content amongst samples A, B and C (raw unfortified *gari*, iron fortified raw *gari* before roasting and iron fortified raw *gari* after roasting *gari* respectively). Also, there were no significant ($P>0.05$) difference in phytate content amongst samples D, E, and (soybean blended *gari*, soybean *gari* iron fortified before roasting and soybean *gari* iron fortified after roasting respectively). The oxalate levels were between 0.199 g/100 g recorded for sample H (oybean flour) and 0.048 g/100 g for sample A (raw *gari* unfortified). Mean values for tannins ranged from 0.0140 g/100 g recorded for sample H (oybean flour) to 0.027 g/100 g recorded for sample B (aw *gari* fortified with iron before roasting). Anti-nutrients have been reported to affect microflora counts in intestines; however they can prevent the bioavailability of certain nutrients [7]. Phytates and tannins are mineral absorption inhibitors, as they form insoluble complexes by binding to minerals which are not readily degraded and bio available in the gastrointestinal tract. Research by Hyojee et al., [22] reported an average of 508.5–1371 mg (thus 0.509–1.371 g) of phytate in legumes. According to Gancheng et al. [15], phytates and tannins contents reduce with processing and heating. The same was observed in the present study as raw soybean flour (sample H) had higher phytate and tannin content as compared to soybean *gari* blends (samples D, E, F). Therefore, using soybean to increase the protein content of gari, the soybean should be mixed with cassava mash before heat treatment.

Elemental composition of iron fortified soybean gari *blend*

Result of the elemental composition of the iron fortified soybean *gari* blends are represented in Table 3.

The highest mean iron value of 1.90 mg/100 g was recorded for sample E (iron fortified soybean *gari* before roasting) and the lowest mean value of 0.86 mg/100 g recorded for sample A (raw *gari* unfortified). The iron content of defatted soybean flour (sample H) was higher than the *gari* samples (Table 3). Even though there were no significant differences ($p>0.05$) in the mean iron values, there was a significant difference between raw unfortified *gari* and the other samples. The *gari* samples fortified with iron before roasting had higher mean value than sample fortified after roasting. The Mg content of soybean flour (sample H) was higher than *gari* samples. The *gari* samples E recorded the highest Mg content of 0.41 mg/100 g (iron fortified soybean *gari* before roasting) and the lowest which was 0.11 mg/100 g recorded for sample A (raw *gari* unfortified). There were no significant difference between Samples B and C (iron fortified *gari* before and after roasting respectively). Samples D and F (soybean *gari* unfortified and iron fortified soybean *gari* after roasting) showed no significant difference. The highest Mg content of soybean flour samples contributed to the high Mg content in the soybean *gari* blends as compared to the samples without soybean blend. A similar pattern was observed for potassium mean values. The potassium content of the soybean flour was 1.49 mg/100 g, samples D, E, F which were all blended with soybean had appreciable levels of potassium as compared to the raw *gari* samples A, B, and C. The zinc levels were quite low for all samples. Minerals are more resistant or stable to manufacturing processes. Samples fortified with iron before heat treatment had higher levels of total iron than samples fortified after heat treatments. Heat treatment is said to improve iron availability and enhances bioaccessibility [23,36]. The highest average temperature level measured during the processing of soybean *gari* blend is 80 °C. This explains why samples B and E (fortified before roasting) had high mean values than C and F (fortified after roasting).

Table 3
Elemental composition of iron fortified soybean *gari* blend.

Sample ID	Elements composition (mg/100 g)			
	Iron	Magnesium	Potassium	Zinc
A	0.86 ± 0.85^{a}	0.11 ± 0.01^{b}	0.40 ± 0.03^{b}	0.03 ± 0.002^{c}
B	1.23 ± 0.07^{abc}	0.16 ± 0.01^{c}	0.62 ± 0.01^{c}	0.01 ± 0.001^{ab}
C	1.03 ± 0.04^{ab}	0.15 ± 0.01^{c}	1.06 ± 0.01^{d}	0.01 ± 0.001^{ab}
D	1.43 ± 0.18^{abc}	0.32 ± 0.01^{d}	1.12 ± 0.01^{d}	0.012 ± 0.006^{b}
E	1.90 ± 0.14^{bc}	0.41 ± 0.01^{e}	1.51 ± 0.01^{f}	0.05 ± 0.001^{ab}
F	1.76 ± 0.15^{bc}	0.33 ± 0.01^{d}	1.23 ± 0.03^{e}	0.04 ± 0.001^{ab}
G	87.02 ± 1.34^{d}	-0.003 ± 0.01^{a}	-0.03 ± 0.01^{a}	-0.004 ± 0.001^{a}
H	2.06 ± 0.22^{c}	0.49 ± 0.01^{f}	1.49 ± 0.04^{f}	0.012 ± 0.001^{b}

Values include means and standard deviation. Means in the same column with the same superscripts are not significantly.
different ($p>0.05$) from each other.
Key
A= Raw *gari* unfortified; *B*= Iron fortified raw *gari* (before roasting); *C*= Iron fortified raw *gari* (after roasting).
D= Soybean *gari* (unfortified); *E*= Iron fortified soybean *gari* (before roasting); *F*= Iron fortified soybean *gari* (after roasting).
G= EDTA Sodium Iron; *H*= Soybean flour.

Table 4
The ratio of phytate to iron and zinc.

Sample code	Phytate:Fe	Phytate:Zn
A	1.61211E-05	0.000462
B	1.12496E-05	0.001384
C	1.34603E-05	0.001386
D	1.93904E-05	0.002311
E	1.45795E-05	0.000554
F	1.57393E-05	0.000693
G	1.99139E-05	0.003419

Key
A= Raw gari unfortified; *B*= Iron fortified raw gari (before roasting); *C*= Iron fortified raw gari (after roasting).
D= Soybean gari (unfortified); *E*= Iron fortified soybean gari (before roasting); *F*= Iron fortified soybean gari (after roasting).
G= FaNa Sodium Iron; *H*= Soybean flour.

The high levels could also be attributed to uniform distribution of the added iron during *gari* processing. The processing of *gari* allows for constant stirring during roasting which allows for uniform distribution of heat to avoid burning. This constant stirring during heating may have allowed for even distribution and binding of the iron to the defatted soybean *gari* blend granules. It could also be deduced that the iron added to the defatted soybean *gari* blend granules after roasting could not uniformly blend with the soybean *gari* granules. This is because the soybean *gari* was in a dried granular form whilst the iron was in a powder form. The differences in particle size can also affect the mixing [31], thereby inhibiting thorough mixing of the roasted soybean *gari* blend and the added iron. According to [38], to ensure uniformity in mixing of samples, the type of mixer, properties of ingredients such as shape, moisture content, flow ability, density of the particles amongst others should be considered. The addition of the defatted soybean flour increased the mineral concentration to appreciable levels as compared to non-blended *gari* samples. Naik & Sekhon [30], also recorded an increase in mineral content with the addition of defatted soybean to pretzel type products.

Estimation of phytate to iron and zinc ratio

The estimated phytate to iron and zinc ratios are presented in Table 4. Values were generated as per the formula described by Gangchemg et al. [15]; Dahdouh et al., [9] as stated under the materials and methods section.

The ratios of phytic acid to iron and zinc contents of the fortified soybean *gari* were below one for all samples. The phytate inhibition effect on iron and zinc is dependant on their molar ratio, and dose/content in the food. Research by Hurrell & Egli, [21] reported that, the bioavailability of iron is affected by a phytate iron ratio of above 1 or even 0.4 for a significant effect on absorption, this present study recorded values that were less than the recommended iron inhibition threshold. This suggests that the fortification of blended *gari* with bio available Ferric Sodium EDTA will be highly absorbed. Low bioavailability of minerals, such as iron and zinc bound to phytic acid can lead to deficiencies of these minerals in human populations, where cereals, grains and legumes are consumed as staple foods [16]. Food processing has shown to reduce the phytate content of food [37], however, combination of different foods in a meal can help in the bioavailability of micronutrients, Madhavan & Little [28], reported that food synergy can help the absorption of micronutrient however this strategy needs to be standardized. Since the estimated anti-nutrient and mineral ratios are within limits, suggesting

Table 5
Effect of fortification on the colour components of iron fortified soybean *gari* blend.

Sample ID	Colour parameters		
	L^*	a^*	b^*
A	71.85 ± 0.01^{E}	0.21 ± 0.02^{B}	15.03 ± 0.01^{F}
B	69.21 ± 0.01^{B}	0.76 ± 0.02^{C}	14.15 ± 0.06^{C}
C	72.82 ± 0.06^{F}	0.13 ± 0.12^{A}	14.35 ± 0.04^{E}
D	69.51 ± 0.01^{C}	1.96 ± 0.02^{F}	14.27 ± 0.01^{D}
E	65.93 ± 0.00^{A}	2.72 ± 0.02^{G}	13.38 ± 0.00^{B}
F	69.84 ± 0.01^{D}	1.85 ± 0.01^{E}	14.13 ± 0.01^{C}
H	72.86 ± 0.01^{F}	1.55 ± 0.02^{D}	12.46 ± 0.01^{A}

LSD: Means in the same column with the same superscripts are not significantly different ($p>0.05$) from each other.
Key
A= Raw gari unfortified; *B*= Iron fortified raw gari (before roasting); *C*= Iron fortified raw gari (after roasting).
D= Soybean gari (unfortified); *E*= Iron fortified soybean gari (before roasting); *F*= Iron fortified soybean gari (after roasting).
H= Soybean flour.
L^* =Lightness; a^*= Redness; b^*= Yellowness.

mineral bioavailabily, this iron fortified soybean *gari* blend can be an appropriate food intervention product in mitigating iron deficiency among women and children in *gari* consuming household.

Colour of iron fortified soybean gari *blend*

Result of the colour analyses of the iron fortified soybean *gari* blend is represented in Table 5 Values in table 5 are mean values and standard deviation of each sample with its mean separation as superscripts for each parameter.

The mean colour values for all the samples were significantly different (Tables 5). The highest L^* (Lightness) mean colour value was 72.82±0.01, whilst the least was 65.93±0.01. The lowest a^* (Redness) mean colour values recorded for the *gari* samples was 0.13±0.12 for Iron fortified raw *gari* (after roasting) and the highest been 2.72±0.02 for iron fortified soybean *gari* (before roasting). The b^* (Yellowness) colour values for iron fortified soybean *gari* blend showed the lowest mean value of 13.38±0.00 and raw *gari* unfortified recorded the highest of 15.03±0.01. Addition of soybean decreased the L^* colour values of *gari*. Also, addition of iron before roasting reduced the L^* mean values of the *gari* samples. However, the addition of iron after roasting improved the L^* colour value of *gari*. Addition of the iron (through fortification) resulted in the increase in the a^* colour values for the *gari* samples. However, b^* colour values for the iron fortified soybean *gari* samples showed a decrease in colour values after the addition of the iron (through fortification). Iron fortification can cause oxidative (redox) reaction in foods [17,18,34]. This oxidative (redox) reaction could make iron fortified foods to discolour and make it unpleasant for consumers. Hurrel [20] and Hurrell & Egli, [21], reported in their study that all iron forms used for fortification has effect on colour and appearance however Ferric sodium EDTA has negative effect on colour. According to Alchar et al., [3], iron is a difficult mineral to be added to food due to its effect on organoleptic qualities of the food and its bioavailability. Addition of iron before roasting the *gari* reduced the L^* and b^* mean colour values significantly while the a^* mean values increased. These results imply that the addition of iron before heat treatment will darken the colour of *gari*.

Conclusion

Blending of cassava mash with defatted soybean flour improved the mineral composition of the resulting soybean *gari* blend; however, fortification with sodium iron increased the iron content significantly. The addition of soybean flour contributed to increasing the content of anti-nutrients in the soybean *gari* blend; however the ratios of phytate to iron and zinc were low and would not inhibit the bioavailability of the fortified iron. Processing/ roasting did affect the content of the fortified iron. The heat and constant stirring of cassava mash during roasting improved the uniform distribution and binding of the fortified iron to the *gari* granules. The colour of the soybean *gari* was affected by the addition of iron, especially for samples fortified with iron before heat treatment. However, fortification of soybean *gari* blend with iron before roasting/heat treatment is recommended. The current study suggests that the commercial food grade defatted soybean flour and ferric sodium FeNa EDTA used in fortifying gari enhanced the protein and iron contents of gari. Iron fortified soybean gari blend developed has the potential to be used as a cheaper food intervention medium in addressing iron deficiency anemia and protein-energy malnutrition among vulnerable groups such as women and children in gari eating households and communities in Africa. It is recommended that further in vivo studies are conducted to determine the bioavailability of the iron from the fortified *gari* on human nutrition and health.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author's contributions

Each author contributed significantly to the research and manuscript writing. Leticia was the PI in the research. She coordinated the whole research and manuscript writing. Fidelis was in charge of data analyses and manuscript writing. Dora prepared the samples for analyses whilst Bernard was in charge of analyses of samples and some data. All authors have proofread the manuscript before been presented for publication.

Data availability

The research team would provide any additional data needed or requested by the reviewers/editor to enrich this manuscript.

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