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Fortification Impact on Nutritional and Anti-Nutritional Composition of Soy-Enriched Gari

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Abstract

This study was carried out to determine and compare the nutritional and anti-nutritional properties of gari enriched with soy curd, residue and control samples. The soy curd and residue flours were used to co-enrich the fermented dewatered and sifted cassava meal during toasting at 10% levels to produce fortified gari samples which were evaluated for nutritional and anti-nutritional properties. The mineral content such as potassium, zinc, calcium, iron and magnesium of formulated samples were higher than those obtained from the control gari samples. The A and B vitamins increased significantly ($P<0.05$) with enrichment but did not follow the same trend in the case of vitamin C which decreased with enrichment. Enrichment correspondingly increased the phytic acid content from 4.12 in the control gari to a range of 5.67-5.76 in the enriched samples, oxalate, condensed tannins and trypsin inhibitors also followed the same trend by increasing with enrichment, while the hydrocyanic acid content decreased from 1.94 mg/g in the control gari to a range of 1.12 mg/g – 1.15 mg/g for the enriched samples. The study concluded that the samples enriched with soy curd and residue were better than the control gari sample in terms of minerals, vitamins A and B, while the anti-nutritional factors were moderate and at a tolerable level. Cassava is not a good source of quality proteins. Therefore, the use of gari as a major staple food in Nigeria should be supplemented with good quality proteins such as soy residue or curd.

Keywords: *Soy Curd, Soy Residue, Cassava, Gari, Anti-Nutrient*

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1. Introduction

Cassava (*Manihot esculanta Crantz*) is a major staple in Nigeria. It is popularly consumed in various forms including gari, fufu and lafun [1]. The cassava root is energy-dense, containing 80% to 90% carbohydrate on a dry weight basis [2] and is a predominantly starchy food [3] grown in more than 90 countries, it also ranks as the 6th most important source of energy in human diets on a worldwide basis and as the 4th supplier of energy after rice, sugar, and corn/maize [4]. However, it is low in protein which stood at 1% to 3% on a dry matter basis [5]. Cassava has the lowest protein/energy ratio of any staple crop; the protein content among common cassava cultivars is typically only 1% [6]. This is of nutritional importance for populations that depend largely on cassava products for their energy needs. An observational study in Kenya and Nigeria [7] showed that consuming cassava as a staple food placed children within the ages of 2 to 5 years old at risk for inadequate protein intake. In addition, cassava contains toxic substances such as cyanide and anti-nutrients such as phytate, nitrate, polyphenols, oxalate, and saponins that can reduce nutrient bioavailability [2, 8]. Hence, improving the nutritional value of cassava food products becomes a necessary intervention in such areas. Nigerian scientists have therefore investigated several ways of enriching cassava products by fortifying with protein rich legumes, chief of which are soybeans. For instance, soybeans have been used to fortify "gari" [9, 10,74] "Tapioca" [11] and "Lafun" [12]; all being widely consumed cassava products. Previous studies have described attempts to improve the nutritional quality of gari using soybeans [13, 14], including storage stability studies, but none has use soy cured and residue to enrich gari in order to increase the nutritional quality. Soy curd and residue contain a reasonable amount of protein, minerals, vitamins and even phytochemicals such as isoflavones which are lacking in the cassava root. Gari can therefore be enriched with soy supplement to improve its nutritional quality. More so, it has been long established that despite high soy nutritional qualities, soybean contains some non-toxic biologically active substances which may inhibit the availability of desired substances or reduce the nutritional value of soybean if not

removed [15]. These include the trypsin inhibitors, hemagglutinin, goitrogens, urease tannin and phytic acid. This study therefore determined the nutritional and anti-nutritional contents, as well as acceptability of the gari enriched with soy curd and residue.

2. Materials and Methods

2.1 Materials

2.1.1 Sources of raw materials

Cassava roots (*Manihot esculenta crantz*) was obtained from the Teaching and Research farm of the Federal University of Technology, Akure, Ondo State, Nigeria. Soybeans (Glycine max (TGX)) were purchased from Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.2 Methods

2.2.1 Soy curd and residue extraction

Soy bean seed (150g) were sorted, cleaned, soaked (12 h) in 2 L of tap water containing 0.5 g $NaHC0_3$ in a cooking pot and boiled for 25 minutes. The boiled and dehulled soybean seeds were then wet milled in a hammer mill. Water was added in ratio 1:8 and a muslin cloth was used to extract the milk (pH 6.40) after which the pH was adjusted to 4.6 by adding 1 Molar citric acid (1 g citric acid to 100 ml of water). The soy milk was allowed to stand and the clear whey at the upper part was decanted while the lower part (curd) was collected after six hours. The residue was obtained after soy milk has been extracted from soy bean mash and filtered. The samples of curd and residue were oven dried (at 60^0C for 24 h), milled, packaged in high density polythene HDPE and stored in the refrigerator for further use. Figure 1 showed the production of the curd and residue.

2.2.2 Gari production and enrichment

The enriched gari sample was produced from cassava tubers using the amended methods of [16] that produced soy-enriched gari using similar methods of enrichment. Cassava tubers were peeled manually with a sharp knife, washed and grated in a locally fabricated mechanical grater which was connected through a belt to a 7 hp driving motor [17]. The mash cassava was packed into Hessian sack and allowed to ferment for 72 hours after which they were pressed in a mechanical press (Addis Engineering Nig. Ltd, Nigeria) to dewater the mash. The dewatered wet cassava cakes were pulverized manually and sifted to remove the fibres. The sifted cassava meal obtained was shared into two portions; one part was roasted and kept as control, while other portion was enriched with soy curd and residue at 10% supplementation levels during toasting [12]. It was then toasted in a wide aluminium pan (called garifier) being heated over wood fire. The toasted soy enriched gari was removed from the iron pot and allowed to cool. The cooled gari samples were then packaged in HDPE film and kept under refrigerated storage until ready for further analysis.

2.3 Analysis

2.3.1 Mineral composition analysis:

Mineral content (sodium, potassium, calcium, magnesium, iron, copper, zinc, manganese and selenium) of the gari samples was determined using an [19] method. Sample was digested with a mixture of concentrated nitric acid, sulfuric acid and perchloric acid (10:0.5:2, v/v) and analysed using an atomic absorption spectrophotometer (GBC 904AA; Germany). The total phosphorus was determined as orthophosphate by the ascorbic acid method after acid digestion and neutralization using phenolphthalein indicator and combined reagent [20]. The absorbance was read at 880 nm (Spectronic 21 D, Miltonroy, New York, USA) and KH_2PO_4 (Merck, Mumbai, India) served as a standard.

2.3.2 Vitamin

The vitamin content of the samples was determined using standard method of [21]. The sample was made to attain the laboratory atmospheric condition on the bench after removing the samples from the storage chamber at less than 4°C. The sample was pressed and completely homogenized in the mortar carefully with pestle to avoid forming balls. A (0.10 g) of the sample was weighed into 10 ml beaker capacity. The sample was extracted in the container by the above stated methods. After the extraction, the extract was concentrated to 1.0 ml for the chromatographic analysis.

2.4 Anti-nutritional analysis

2.4.1 Estimation of phytic acid

This was determined using [22] method. Four gram (4 g) of gari sample was soaked in 100 ml of 2% hydrochloric acid for 3 hrs and then filtered. 5 ml of 0.3% ammonium thiocyanate solution was added to 25 ml of the filtrate. Also added to the mixture was 53.5 ml of distilled water. This was then titrated against a standard iron (III) chloride solution until a brownish yellow colour persisted for 5 min. The phytate content was calculated from the iron determinations, using a 4:1 iron-to-phytate molecular ratio.

2.4.2 Determination of tannin content

This was done using the method of Meduoa [23]. Two gram (2 g) of each sample was weighed into a 250 ml flask followed by addition of 200 ml of 0.004 $MK_3Fe\ (CN)_6$ and 10 ml of 0.008 M $FeCl_3$ in 0.008 M HCl. The flask was allowed to stand for 20 mins and stirred occasionally at 10 mins interval and 1 ml aliquot was removed. This aliquot was added 2 ml of 0.008 M $FeCl_3$ in 0.008M HCl and 10 ml of 0.0015 $MK_3Fe(CN)_6$. After adding the final reagent, the absorbance was then read at 720 nm after 30 seconds against a blank.

$$Tannin\ \left(\frac{mg}{100g}\right) = \frac{Absolute\ of\ the\ sample\ x\ concentration\ of\ the\ standard\ x\ DF}{Absorbance\ of\ the\ standard\ x\ sample\ size}$$

Where Df = Dilution factor

2.4.3 Determination of phytate

The gari sample was done using the method described by [24]. About 8 g of gari samples was dispersed in 200 ml of 2% HCl and extracted. Following extraction, the dispersion was filtered and 50ml of the filtrate was mixed with 10 cm^3 of 0.3% ammonium cyanide (NH_4SCN) and diluted with 107 ml of distilled water. The extract was titrated against 0.00195g/ml of Ferric chloride solution until a brownish yellow colour persisted. Phytate content was estimated with the expression:

$$Phytate\ Phosphorous = Iron\ equivalent\ x\ 1.95\ g\ of\ titre$$
$$Phytate = Phytate\ Phosphorus\ x\ 3.65$$

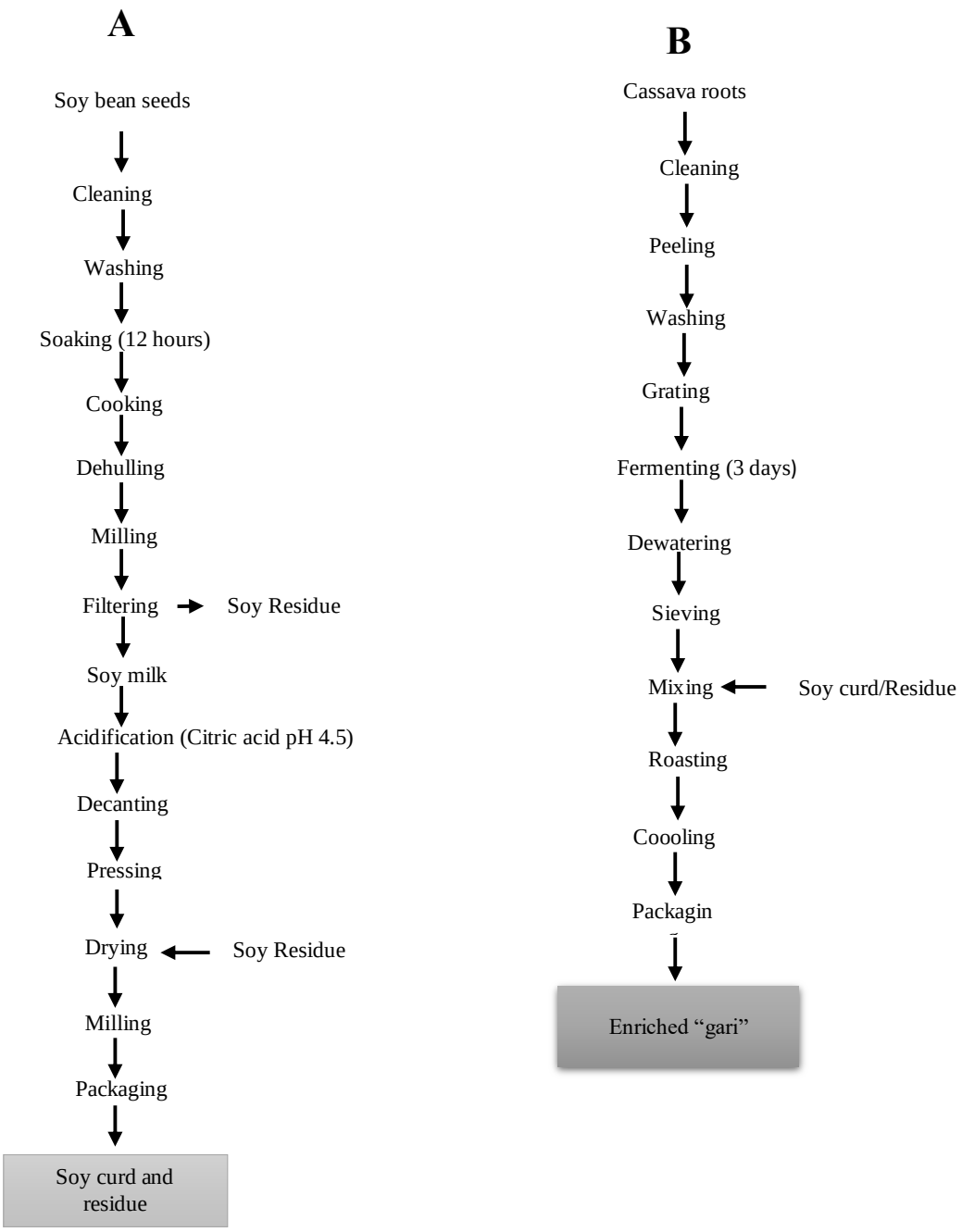

Figure 1: Production of soy supplement (curd and residue) (A) and enriched "gari" samples (B)
Source: Osuji and Anyaiwe (2010)

2.4.4 Determination of oxalate

The method described by [25] was used. About two gram (2 g) of gari sample was digested with 10 ml 6M HCl for one hour and made up to 250 ml in a volumetric flask. The pH of the filtrate was adjusted with conc. NH_4OH solution until the colour of the solution changed from salmon pink colour to a faint yellow colour. Thereafter, the filtrate was treated with 10 ml of 5 % $CaCl_2$ solution to precipitate the insoluble oxalate. The suspension was centrifuged at 2500 rpm, after which the supernatant was decanted. The precipitate was dissolved in 10 ml of 20 % (v/v) H_2SO_4 and the solution was made up to 300 ml. An aliquot (125 ml) was heated until near boiling point and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink colour which persisted for about 30 seconds after which the burette reading was taken and used to estimate the oxalate content.

$$N = \frac{Titre\ value\ x\ 0.0025\ x\ DF}{5}$$

$$Oxalate\ (\%) = \frac{N}{Sample\ size} x\ 10$$

Where Df = Dilution factor; 0.0025 = Volume of KMnO4

2.4.5 Trypsin inhibitor

The analysis of trypsin inhibitor was carried out according to the method described by [26]. About 1g of the sample was weighed into a beaker containing a magnetic stirring bar, before 50ml of sodium hydroxide solution was added and the

suspension was agitated slowly. After 3 hrs, the pH was measured; pH ranged between 8.4 and 10.0. An aliquot of suspension were taken with a pipette and diluted with distilled water so that the sample trypsin inhibitor concentration was sufficient for 40-60% trypsin inhibitor. When it is not possible to estimate the expected trypsin inhibitor units, more than one dilution will be made. With serological pipettes, 0, 0.6, 1.0, 1.4 and 1.8 ml of the diluted suspension which was added to duplicate sets of test tubes. Water was added to bring the volume to 2 ml in each tube, with a regular time interval for the different tubes. 2 ml trypsin solution was added to each tube and quickly mixed on the Vortex stirrer and placed in the 37ºC water bath. 5 ml BAPNA was added to each tube, mixed on Vortex stirrer. The samples were incubated for 10 min at 37ºC. After exactly 10 min, the reaction was stopped by addition of 1 ml acetic acid solution followed by mixing on the Vortex stirrer. Blank samples were prepared as above, except that trypsin was added after acetic acid. The contents of each tube were filtered and absorbance was measured at 410 nm. One trypsin unit is arbitrarily defined as the amount of enzyme, which will increase absorbance at 410 nm by 0.01 units after 10 mins of reaction for each 10 ml of reaction volume.

2.4.6 Determination of cyanogenic glucoside

The method of [27] was used for the determination of cyanogenic glucoside in the gari ample. Extraction of cyanide: About (5g) was weighed into a conical flask and 50 ml of distilled water was added after which the flask was corked. The mixture was allowed to stand overnight. The extract was then filtered and used for glycoside determination. Filtrate (1 ml) from each sample extract was transferred into test tubes and 4 ml of alkaline picrate was added to each tube, and the tubes were placed in a water bath for 5 mins. Colour development (reddish brown) occurred after which the absorbance was read in a spectrophotometer (model: Jenway 6305) at 720 nm. The blank solution was prepared using 1 ml distilled water and 5 ml alkaline picrate solution. The concentration of glycoside was determined using a standard.

$$Conc.\,of\ glycoside = \frac{Absorbance\ of\ the\ test\ sample\ \ x\ concentration\ of\ standard}{Absorbance\ of\ standard\ x\ weight\ of\ sample}$$

3. Results and Discussion

The present study considered the nutritional deficiency in gari by formulating an enriched gari product using soy curd and residue.

3.1 Mineral Composition of Gari Samples

The mineral composition of formulated enriched food samples (Table 1) showed that potassium was the most abundant in enriched and control samples, which make the observation similar to other findings like [28, 29] who reported potassium to be the most abundant mineral in Nigerian agricultural products. These also agreed with the findings of [30] that listed potassium as the most readily available mineral constituent in most foods. Potassium helps the kidney to function normally and control blood pressure [31]. Iron (Fe) and copper were ranged 0.21-0.23 mg/100g and 1.45-3.16 mg/100 g respectively.

Table 1: Mineral (mg/100g) composition of gari enriched with soy curd and residue

Sample	GIC	GEC	GMR	GMC	REF
Ca	9.78±0.06^{e}	9.18±0.19^{e}	46.24±1.11^{d}	66.54±0.40^{c}	
Mg	17.75±0.11^{e}	17.53±0.05^{e}	74.09±0.19^{d}	84.63±0.01^{c}	
Na	11.24±0.03^{c}	11.26±0.00^{c}	13.28±0.01^{b}	8.82±0.05^{d}	
K	182.66±2.74^{f}	188.57±0.17^{e}	195.27±1.01^{d}	625.10±2.07^{b}	
Fe	0.21±0.01^{e}	0.23±0.06^{ef}	1.45±0.01^{d}	3.16±0.00^{c}	
Zn	0.28±0.01^{e}	0.26±0.01^{e}	0.64±0.02^{d}	1.39±0.01^{c}	
Mn	0.36±0.00^{e}	0.30±0.01^{ef}	0.48±0.00^{d}	1.17±0.05^{b}	
Cu	0.46±0.00^{b}	0.34±0.00^{b}	0.85±0.01^{d}	0.82±0.00^{c}	
P	105.02±1.06^{ef}	107.94±1.6^{e}	136.87±0.35^{d}	186.25±5.14^{c}	
Pb	ND	ND	ND	ND	< 0.1
Cd	ND	ND	ND	ND	< 0.1
Ca/P	2.00	2.09	3.24	4.64	> 1
Na/K	0.03	0.04	0.07	0.08	< 1

Data represent mean ± standard deviation of three replicates, values with different superscripts along the same column are significantly different ($p<0.05$).
Key: GIC = Control sample, GEC= commercial sample GMC, = 'gari' enriched with 10% curd, GMR= 'gari' enriched with 10% residue *REC - FAO/WHO 2000.

The calcium content of the gari samples ranged from 46.24 to 66.54 mg/100g in the enriched sample and significantly decreased ($p\leq0.05$) in the control samples which ranged from 9.18 to 9.78 mg/100g respectively. The result showed that the enriched samples are better source of minerals compared to the commercial and control samples. This agreed with the findings of [31]. The high level of calcium in enriched samples would promote good bone formation, tooth health for adults and children who consume the products [32]. The findings also corroborated with the findings of [32] which reported that soy-bean is a rich source of vitamins and minerals. Samples enriched with soy curd and residue showed product with similar trend on copper, magnesium, zinc, manganese and phosphorus which is significantly ($p\leq0.05$) different from the control and commercial samples. The calcium: phosphorous ratio (Ca/p) of the enriched products which was represented as 3.24 to 4.64, were observed to be higher than the control samples which ranged from 2.00 to 2.09. The values obtained were higher than the recommended value of 1.0 [33]. This inferred that the diets are suitable to provide the required calcium and phosphorous for the formation of bones and teeth, as well as controlling the level of calcium in blood of the consumers [33, 34]. Sodium/potassium (Na/K) ratios of the enriched products 0.07 to 0.08 were significantly ($p\leq0.05$) higher than the value obtained from the control samples which ranged from 0.03 to 0.04. However, these

values were below the recommended value of [35]. Because of Na/K low value, it is expected that the formulated food samples will be suitable for consumers who use 'gari' as their staple food. A high intake of potassium has been reported to protect against increase in blood pressure and other cardiovascular risks [36]. Hence, the sodium to potassium (Na/K) ratio in the body is of great concern for the prevention of high blood pressure. A Na/K ratio less than one is recommended in the diets of people who are prone to high blood pressure and similar for children with immature heart [37, 38].

3.2 Vitamin Composition of Gari Samples

The vitamin composition of the gari samples was presented in Table 2. From the results obtained, significant differences ($p < 0.05$) existed among the Vitamin A samples which ranged from GIC 6.45mg/100g, GEC 6.65mg/100g for the control samples and GMR 7.89mg/g and GMC 7.94mg/100g for the enriched samples respectively. Sample (GMC) had the highest value of vitamin A when compared with other samples but slightly different from the sample enriched with residue (GMR). The enrichment gari products were observed to have highest vitamin B_1 content (1.07-1.05mg/100g) than the control (1.27-1.37mg/100g). According to [39], the recommended dietary allowance (RDA) of vitamin B_1 for adult is 1.2mg/100g and only the enriched products met this requirement. Vitamin B_1 (thiamine) is a part of the co-enzyme, thiamine pyrophosphate (TPP) that plays critical role in carbohydrate metabolism (breakdown of glucose for energy). It also acts as coenzyme in the metabolism of the amino acids leucine, isoleucine and valine. Deficiency in thiamine results in beriberi [40]. The enriched gari products were also observed to have been significantly ($p < 0.05$) higher in vitamin B_2 (5.25-6.10 mg/100g) than the control (4.42–4.14 mg/100g) samples. [39] Placed the recommended dietary allowance (RDA) of vitamin B_2 for adult at 1.3 mg/100g. All the samples met this requirement. Vitamin B_2 (riboflavin) is a part of the coenzymes, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD), needed for oxidation/reduction reactions and also involved in energy production. Its deficiencies results in cracks and sores around the mouth and nose, sore throat, magenta tongue and skin rash among others [40]. The value of vitamin B_3 ranged (9.09-8.64mg/100g) for the control samples and (8.22-8.33mg/100g) in the samples enriched with soy supplement. The control samples were slightly higher than the enriched sample which inferred that the enrichment does not have any significant influence on the samples. According to [39], the recommended dietary allowance (RDA) for adult is 16 mg/100g, this means that none of the samples met the recommended dietary allowance (RDA) for vitamin B_3 (niacin). Vitamin B_3 is a part of the coenzyme, nicotinamide adenine dinucleotide (NAD) or its phosphate form, NADP plays a key role in energy production, synthesis of fatty acids and steroids. The coenzyme is also involved in DNA replication, repair, and cell differentiation. Deficiency of this very important vitamin leads to pellagra [40]. The observed increase in vitamin B in the enriched samples are in agreement with the report of [41] which observed the increase in vitamin B concentration of bambara fortified maize sorghum mix. It was also observed that sample GMC had the highest value of vitamin A, B1, B2 and B3 which is significantly ($P \leq 0.05$) different from other samples, while the control samples are lowest in value which could be due to absence of soy supplementation [32]. Vitamin B4 did not maintain any trend rather, a slight different was observed between the sample enriched with soy curd 3.50mg/100g and soy residue 1.39mg/100g. This was slightly different from commercial GEC 1.17 and control GIC 1.30mg/100g sample respectively. Significant difference ($P \leq 0.05$) was observed in vitamin B5 which ranged from 1.02 to 1.06 in the control samples and 1.90 to 4.65 in the enriched samples, with the sample enriched with residue having the highest value. In vitamin B6, there was no significant noticeable difference among the samples. Vitamin B9 did not show any noticeable difference between the control samples (GIC 2.16 – GEC 2.23) rather, value obtained was slightly higher than the enriched samples GMR 2.00 and GMC 2.08. It was also observed in vitamin B12 that control samples GIC 5.59 and GEC 5.49 exhibited no significant difference, but was slightly higher in value than the enriched samples (GMR 4.87 and GMC 5.55), which might inferred that the enrichment applied could not impact on this particular vitamin. The vitamin composition of the gari samples investigated in this study was comparable to that of two Japanese sweet potatoes varieties in vitamin B1 (0.05-0.13 mg/100 g), B2 (0.04-0.06 mg/100 g), B6 (0.04-0.11 mg/100 g) and niacin (0.63-0.91 mg/100g) contents [42]. The value obtained for Vitamin C in table 2 ranged GMR 10.46 to GMC 10.89 for the enriched samples and GEC 14.08 to GEC 14.50 in the control samples. The enriched samples have lower value which could be attributed to higher temperature during processing [43]. Cassava had been reported to contain ascorbic acid of about 120mg – 150mg/100g on a wet basis and this value reduced to about 25 – 30 % of the original value in 5 days and subsequently dropped to insignificant values after 8 days [44].

Table 2: Vitamin (mg/100g) composition of gari enriched with soy curd and residue

Sample	GIC	GEC	GMC	GMR
VIT A	6.45 ± 0.01^{c}	6.65 ± 0.00^{b}	7.89 ± 0.06^{a}	7.94 ± 0.00^{a}
VIT B1	1.07 ± 0.01^{d}	1.05 ± 0.01^{de}	1.07 ± 0.00^{d}	1.17 ± 0.00^{c}
VIT B2	4.42 ± 0.06^{b}	4.14 ± 0.00^{c}	6.10 ± 0.00^{a}	1.01 ± 0.00^{f}
VIT B3	9.09 ± 0.00^{a}	8.64 ± 0.01^{b}	8.22 ± 0.05^{c}	8.33 ± 0.03^{f}
VIT B4	1.30 ± 0.04^{d}	1.17 ± 0.01^{e}	9.39 ± 0.00^{a}	9.50 ± 0.00^{a}
VIT B5	1.02 ± 0.01^{e}	1.06 ± 0.01^{e}	0.64 ± 0.02^{d}	4.65 ± 0.01^{b}
VIT B6	1.12 ± 0.02^{d}	1.13 ± 0.02^{d}	1.05 ± 0.00^{e}	1.23 ± 0.02^{c}
VIT B9	2.16 ± 0.02^{b}	2.23 ± 0.01^{a}	2.00 ± 0.00^{d}	2.08 ± 0.01^{c}
VIT B12	5.59 ± 0.05^{a}	5.49 ± 0.00^{bc}	4.87 ± 0.06^{e}	5.55 ± 0.06^{b}
VIT C	14.50 ± 0.01^{a}	14.08 ± 0.02^{b}	10.46 ± 0.00^{d}	10.89 ± 0.01^{c}

Data represent mean ± standard deviation of three replicates, values with different superscripts along the same column are significantly different ($p<0.05$) KEY GIC = Control sample, GEC= commercial sample GMC, = 'gari' enriched with 10% curd, GMR= 'gari' enriched with 10% residue *REC - FAO/WHO 2000.

3.3 Antinutrients

The effect of enrichment on the antinutritional factors of gari is presented in table 3. All the antinutritional factors analysed; alkaloids, phytate, oxalate, tannins and hydrocyanic acid decreased with fermentation of the gari mash and heat process applied. Phytate content of the gari samples reduced significantly ($p<0.05$) from 5.76mg/g in the enriched samples to 3.48mg/g in the control sample. The result agreed with the earlier report of [45, 46] on a decreased in phytate content of cocoyam tubers from 855mg/g to 13mg/g with enrichment. This finding is also in consistent with the results of [47, 48] who reported 96.3-54.77% reduction in phytic acid content of peanut and soy bean respectively. Phytates are quite high in soybean and are considered beneficial due to its anticancer properties and capability of preventing heart diseases [49]. The reduction may be attributed to the activity of the endogenous phytase enzyme from the raw ingredient and inherent microorganisms which are capable of hydrolysing the phytic acid in the fermented food during preparations into inositol and orthophosphate [50, 51]. The residual phytate content of gari samples fall within the recommendation of [52] that phytates should be lowered as much as possible, ideally to 25 mg or less per 100g or to about 0.03% of the phytate-containing food eaten. At this level, micronutrient losses are minimal because retention of phosphorus decreases when phytate in food is 30 – 40% or more of total phosphorus [53].

Table 3: Anti-nutritional properties of gari enriched with soy curd and residue

Samples	Phytate (mg/g)	Oxalate (mg/g)	Hydrogen Cyanide (mg/g)	Tannins (mg/g)	Alkaloid (mg/g)	Trypsin Inhibitor (mg/g)
GIC	4.12 ± 0.00^{b}	0.09 ± 0.00^{c}	1.76 ± 0.00^{a}	2.00 ± 0.00^{bc}	24.54 ± 0.01^{c}	14.28 ± 0.00^{b}
GEC	3.48 ± 0.15^{c}	0.11 ± 0.01^{b}	1.94 ± 0.00^{a}	1.19 ± 0.00^{d}	25.86 ± 0.00^{c}	13.53 ± 0.07^{b}
GMC	5.67 ± 0.01^{a}	0.18 ± 0.00^{a}	1.12 ± 0.01^{b}	2.82 ± 0.00^{b}	47.39 ± 0.02^{a}	27.12 ± 0.00^{a}
GMR	5.76 ± 0.01^{a}	0.18 ± 0.01^{a}	1.15 ± 0.01^{b}	3.64 ± 0.01^{a}	34.32 ± 0.01^{b}	28.74 ± 0.34^{a}

Data represent mean ± standard deviation of three replicates, values with different superscripts along the same column are significantly different ($p<0.05$) KEY GIC = Control sample, GEC= commercial sample GMC, = 'gari' enriched with 10% curd, GMR= 'gari' enriched with 10% residue *REC - FAO/WHO 2000.

Oxalate content of the control gari samples showed a significant difference ($P<0.05$) when compared with the enriched samples which is obtained as 0.09mg/g - 0.11mg/g in the control samples and 0.18mg/g to 0.28mg/g in the enriched samples. The oxalate values observed were generally low and might not affect the bioavailability of calcium; this could be due to the processing method applied. The value obtained was lower than cowpea (7.34 mg/g) reported by [54]. It is known that oxalate forms insoluble complex with calcium ions and often anticipated that oxalate containing foods when consumed may interfere with calcium metabolism. However, [55] and [56] have shown that the risk for calcium deficiency due to oxalate rich plants is very minimal.

Table 3 also shows some significant increased ($p<0.05$) in condensed tannins which ranged from 1.19-2.00 in the control samples to 2.82-3.64 in the enriched samples. The low tannin content of the 'gari' samples could be responsible for the absence of bitter taste in the 'gari' products [43]. The reduction of tannins in the dried 'gari' samples could be also due to tannins being thermally labile and sensitive to oxidation [12]. [57] Observed that the decreased in tannin content may be due to binding of polyphenols with other organic substances and protein or alteration in the chemical structure of polyphenols. Both processing and drying showed significant reduction in tannin content making them a good method for the reduction of anti-nutritional factors [55]. In this regard, this product is assumed to be safe since the tannin content is far below the detrimental dose of 0.7 – 0.9% [58]. Tannins are dietary phytochemicals that are responsible for the astringent taste of foods and drinks [59]. The presence of tannins can cause browning in fresh foods and processed products which would affect the nutritive value of food by forming a complex with protein (both substrate and enzyme) thereby inhibiting digestion and absorption [58]. They also impart dull colour on cassava products and this affect the market value of the products [12]. Tannins are another group of anti-nutrient observed to reduce the absorption of minerals such as iron and copper however, the chelating of these metals could be beneficial as this is one of the mechanism by which phenolic compounds exert antioxidant properties [58].

Hydrocyanic acid content of the gari samples decreased significantly from 1.76mg/g - 1.94mg/g in the control gari samples to 1.12 -1.15mg/g in the enriched gari samples. The decreased in cyanide content could be due to hydrolysis by fermenting microorganisms or processing temperature [9]. The residual cyanide in the gari samples was much lower than the recommended safe level of 10mg set by regulatory bodies. This reduction in residual cyanide is in agreement with similar report of [60] that 70 – 75% reduction in cyanogenic glycosides content of fermented roots and leaves of cassava. The hydrocyanic acid contents observed was generally low which could be attributed to fermentation process carried out, dilution effect of gari by the soy supplement and heat involved during garifying [9]. Observation also reported that hydrogen cyanide was lower than the recommended values of 20.0 mg/kg [61, 62] and the lethal dose of 40-60 mg/kg suggested for adult [63]. It was also lower than the value of 38.00 – 32.00 mg/kg reported in earlier studies by [63, 64]. The lower value recorded in the enriched samples could be due to dilution effect of the soy protein in the supplement as observed in soy enriched gari by [66, 14, 62, 9] which recommended safe level of 20 mg/kg. The value obtained was also much lower than the recommended safe level of 10mg/g set by regulatory bodies. This observation is also in line with that of [67] confirming fermentation to be a very effective process for eradication of endogenous cyanic compounds in cassava roots. Moreover, the cyanide levels are far below the detrimental level of 30 mg/kg reported by [68] and similar to the report of other investigators [69, 70] 3.96 mg/g, 5.13 mg/g and 5.23 mg/g in that order respectively. This inferred that the gari products

could therefore be considered safe with regards to cyanide poisoning.

Alkaloids content of the gari sample differed significantly ($p<0.05$) with enrichment which ranged from 24.54 -25.86 in the control samples to 34.32-47.37 in the enriched samples. The result obtained is in agreement with previous literature report that tubers and plant leaves contain a substantial proportion of alkaloids [44, 72].

The mean values for Trypsin Inhibitor Units (TIU) ranged from 13.53-14.28 in the control samples to 27.12-28.74 in the enriched samples. Trypsin inhibitor content was observed to have increased with supplement and this suggests that soy bean contain trypsin inhibitors. Although, its activity was low when compared with the value (8.34%) reported by [50] in soy enriched cassava flour, 5.56% by [9] for soy-melon supplemented gari. Soy bean contains anti-nutritional factors such as trypsin inhibitors, phytic acid and saponins which decreased their nutritive value of (legumes) and can cause health problems to both human and the animals when taken in a large quantity [73]. But in the other way, [49] reported that protease inhibitors appear to have an anti-carcinogen mechanism of action whereby they prevent normal functioning of proteins involved in the activation of certain cancer related genes. Large amounts prevent proper digestion of soy bean; hence, most processed soy foods contain only 5% or less of the naturally occurring protease inhibitors.

However, [74] reported that trypsin inhibitors activity of the average British diet was calculated as shown to be 330mg/person/day. Since small amounts of protease inhibitors do not cause growth suppression but do have anticancer effects in animals, soy foods can be consumed safely without fear of growth suppression [49].

4. Conclusion

Incorporation of soy curd and residue flour into gari had varying effects on the nutritional and anti-nutritional properties of the products. Soy enrichment resulted in improving the nutritional composition in terms of minerals, vitamin and thus reduced the anti-nutritional properties to a minimal level. Further studies are necessary to determine protein digestibility and microbial examination. While stability studies should be carried out on soy enhanced gari products to determine their safety and shelf-life. Fortification of gari can also be carried out by the use of soybean meal, soy protein isolate or concentrates in varying substitution levels. Soy enriched gari samples had a low level of anti-nutritional components, making them safe for consumption. Therefore, gari could be fortified with soy (curd or residue) to alleviate malnutrition problems caused by root crops

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