



Effects of Enrichment on Amino Acid Profile, Mineral Composition and Anti-Nutritional Factors of *Lafun* Powder

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ABSTRACT

“Lafun” was enriched with soy protein supplements (curd or residue) during the mashing before drying at 10%. The amino acid result indicated that enrichment improved the amino acids contents as well as the protein quality of the products. While enrichment generally improved the mineral element composition, enrichment with the residue significantly improved the mineral composition when compared to enrichment curd. That the products were hygienically produced was confirmed by the non-detection of heavy metals which may suggest its safety for human consumption. Condensed tannins, phytate, oxalate, hydrogen cyanide and trypsin inhibitor were tested as possible anti-nutritional factors in the product and the values obtained were within safe level and correlated with those reported in previous or similar studies. It was concluded that enrichment with soy curd/residue is a safe and viable means to improve the nutritional benefits derivable from “Lafun” especially for those that “Lafun” is a major staple.

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Introduction

Cassava (*Manihot esculenta* Crantz), though originated from South America (Olanrewaju and Idowu, 2017) is now a common staple in many countries including Nigeria (Okpako et al., 2008). Globally, its importance is rated sixth in terms of annual production (Burns et al., 2012) and Nigeria is considered the largest producer worldwide (Omolara, 2014). The processing vary from location to location but the commonly available forms in Nigeria and in order of popularity and acceptability are *gari*, *fufu* and *lafun* (Adebayo-Oyetoro et al., 2017; Okafor et al., 2017). Besides this, cassava tubers can also be boiled and eaten (Oluba et al., 2018). Apparently, with up to 90% carbohydrate on dry weight basis, cassava is mainly a carbohydrate and an energy dense food. The tuber is estimated to be the source of energy for more than 800 million people (Burns et al., 2012; Samuel et al., 2012).

Cassava is nutritionally deficient in many vital nutrients essential for growth and development, especially in children and young adults (Isaac-bamgboye et al., 2020). This may be responsible for the observed stunted growth in people whose staples are mainly cassava and cassava products. Cassava is deficient in protein, mineral and

vitamin (Isaac-bamgboye et al., 2020). Previous study reported that the crude protein contents of most cassava foods ranged between 1 and 3% on dry weight basis (Isaac-bamgboye et al., 2020). Obviously, this is too poor and the impact of such nutritionally deficient food may be significant for populations, especially children that largely depend on cassava products considering the fact they derive most of their daily nutritional needs from cassava and its products (Samuel et al., 2012).

Among the various products of cassava commonly consumed in Nigeria, “Lafun” is especially common in the South Western part of Nigeria and specifically, mostly consumed by the Yoruba tribe (Sawyer et al., 2018). “Lafun” is a flour obtained from fermented, dried and milled cassava tubers. Usually, after harvesting, the tuber are peeled, soaked in water for 2-3 days for fermentation and softening of the tubers. Thereafter, they are sun-dried, milled, sieved and packaged as “Lafun” flour. The flour is made into a dough meal by mixing with hot water and usually consumed with soups (Taiwo et al., 2016; Adebayo-Oyetoro et al., 2017; Sawyer et al., 2018).

A common challenge with cassava and its products is the presence of anti-nutritional factors such as cyanogenic glycosides which is toxic and could lead to food poisoning in consumers if not well processed (Adebayo-Oyetoro et al., 2017; Sawyerr et al., 2018). However, common processing techniques such as peeling, chopping, soaking, fermentation, dewatering, frying and sun drying had been reported to reduce the contents of the anti-nutritional factors (Kemdirim et al., 1995; Sawyerr et al., 2018; Ndam et al., 2019). The potentials of different processing techniques to reduce the cyanide contents of cassava had been reported (Ndam et al., 2019). According to the authors (Ndam et al., 2019), the cyanide contents of the different cultivars (fresh) evaluated ranged from 61 - 118 and 79 - 181 ppm for improved and local cultivars, respectively. According to them, cutting caused toxicity reduction of 47 and 48% for improved and local cultivars. The highest toxicity reduction obtained by them was 81 and 79% for "fufu", a product of wet fermentation, for improved and local cultivars. Other studies had also confirmed the potentials of processing to reduce the contents of hydrogen cyanide in cassava products (Laya et al., 2018). Higher temperature, such as often traditionally used for frying *gari* had been reported to cause little reduction in cyanide contents of *gari* - a cassava product obtained by high temperature frying. This was reported to be due to the inactivation of the linamarase enzyme which is responsible of the hydrolysis of cyanogenic glucoside (Kemdirim et al., 1995).

In order to improve the nutritional composition of cassava and cassava products, various efforts had been made to fortify cassava or supplement cassava products with other food items of nutritional significance. Studies on the tuber had included bio-fortification of some cultivars with beta carotene (pro-vitamin A) and are able to provide up to 40% of the recommended daily allowance of vitamin A for children (Oluba et al., 2018). Soy beans (*Glycine max*) is a notable high protein legumes with considerable quantity of other nutrients (vitamin, minerals) and non-nutrient phytochemicals (e.g isoflavones) (Samuel et al., 2012). Soy beans have been used to supplement different cassava products (Okafor et al., 2017). Other food items used to supplement cassava products include African yam bean (Isaac-bamgboye et al., 2020). There had also been the production of other acceptable nutritionally enriched food (e.g chips) that can be stored at home (Taiwo et al., 2016; Okafor et al., 2017; Isaac-bamgboye et al., 2020).

Enrichment of nutritionally deficient food item with other essential nutrients is an innovative means practised globally to improve the nutritional benefits derivable from the enriched foods more so when such food is a commonly consumed food item within a region. Therefore, this study was aimed at enriching "Lafun" with soy curd or residue and studying the effect of the enrichment on some essential nutritional profiles of the foods - amino acid, mineral elements compositions and anti-nutritional factors.

Materials and Methods

Sources of Raw Materials

Cassava roots (*M. esculenta* crantz) were obtained from the Teaching and Research Farm of the Federal University of Technology, Akure, Ondo State, Nigeria. Soybean

(*Glycine max* (TGX)) was purchased from Michael Okpara University of Agriculture (UMUDIKE), Otu, Abia State, Nigeria.

Soy Curd and Residue Extraction

Soy bean seed (150 g) were sorted, cleaned, soaked (12 h) in 2 L of tap water containing 0.5 g NaHCO₃ in a cooking pot and boiled for 25 min. 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) and the residue was kept separate. Thereafter, the pH of the extracted milk was adjusted to 4.6 by adding 1 M citric acid. 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 curd and residue were oven dried (at 60 °C for 24 h), milled, packaged in high density polyethylene HDPE and stored in the refrigerator until needed for further use. Figure IA shows the production chart for the curd and residue.

"Lafun" Production and Enrichment

Freshly harvested cassava roots were peeled with knife, washed and cut into chunks, fermented for 4 days (pH 3.67), washed, sifted, milled into pulp and divided into two portions (Figure IB). One portion was used as control (CL) while the other portion was enriched with either dry soy curd or residue using Pearson scale with, 10% enrichment level and also taking into consideration the water content of the mash at 100%. Sample supplemented with curd was named "Lafun enriched with curd" (LEC) and the other sample "Lafun enriched with residue" (LER). A commercial "Lafun" sample (CS) was obtained from FIRO Oshodi, Lagos for comparison.

Amino Acid Analysis

The sample of 2.0 g was weighed into the 250 mL conical flask capacity. The sample was defatted by extracting the fat content of the sample 30 mL of the petroleum spirit three times with soxhlet that was equipped with thimble. The amino acid content of the sample was recovered by extracting with 30 mL of the dichloromethane three times before concentrating to 1.0mL. The concentrated extract was derivatised for volatility that is suitable for Gas chromatography analysis (GC) system (Agilent6890NNetwork) connected to a mass selective detector (Agilent5973Network).

Mineral Analysis

About 0.1 g of the ashed sample was weighed into the pre cleaned borosilicate 250 mL capacity beaker for digestion. Thirty milliliters (30 mL) of the nitric acid was added into the weighed sample in the beaker. The sample with the digesting solvent was placed on the hot plate before digestion in the fume cupboard and the beaker along with its contents after the digestion were allowed to cool. The digested samples were measured into pre-cleaned borosilicate glass containers before the analysis by Atomic Absorption Spectrophotometer. Standards of Manganese, Zinc, Copper, Iron, Calcium, Magnesium, Potassium and Sodium solutions of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/L were made from the each of the heavy metals solution (1 g/L stock solutions) of the analyses.

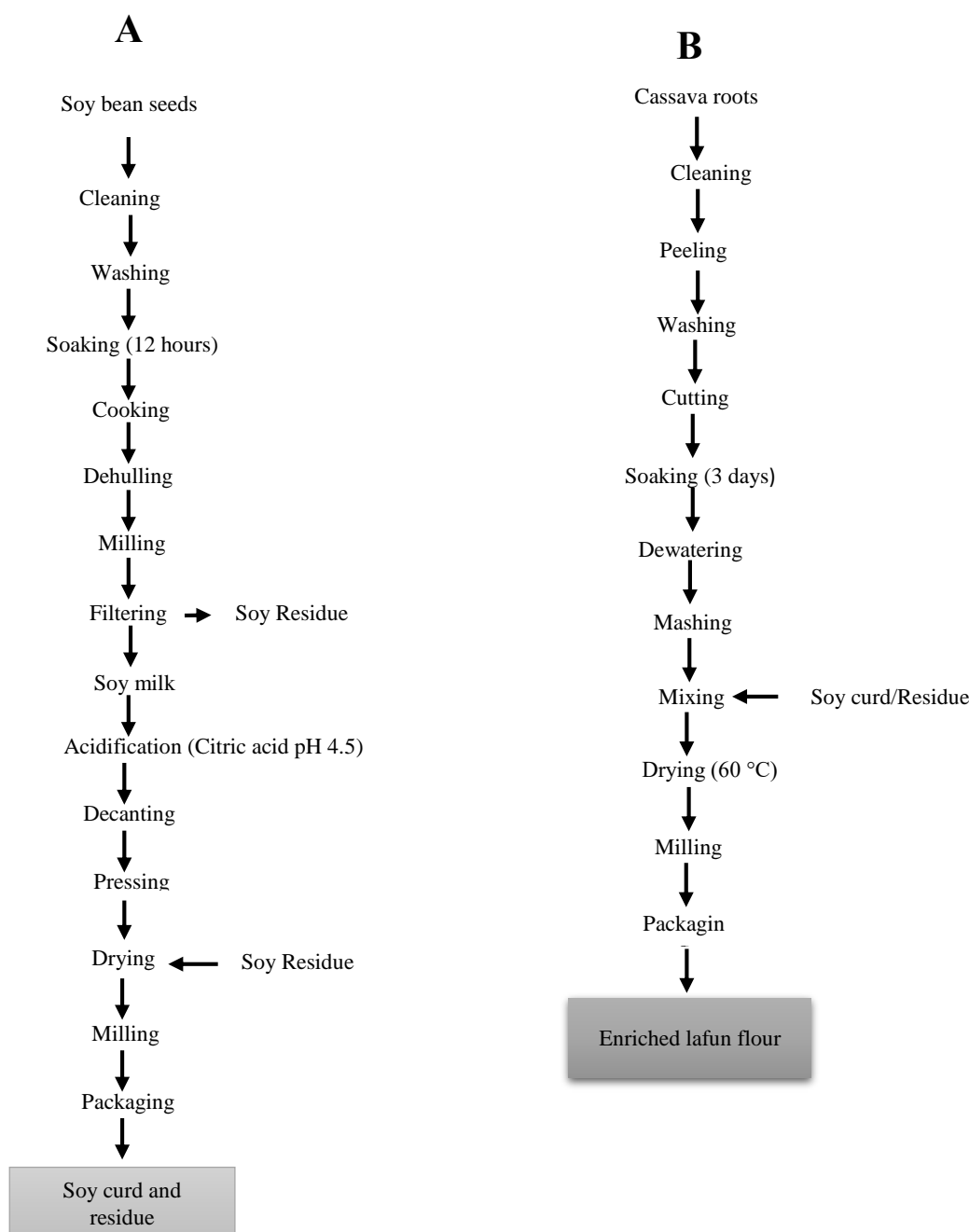


Figure 1. Production of soy supplement (curd and residue) (A) and enriched lafun samples (B)

The filtrate of the digested samples and standard solutions were evaluated with AAS and metal detection limit in the sample was 0.0001mg/L. Manganese, Zinc, Copper, Iron, Calcium, Magnesium, Potassium and Sodium cathode lamps were used for the analysis of the respective mineral ions in the standards and the filtrate of the samples. The flame was generated with gas mixtures.

Determination of phosphorus

About 25 mg of the sample was weighed into the Schoniger flask and burnt in excess of oxygen gas. The product was digested with nitric acid. The content was boiled for a minute to ensure complete conversion of phosphorus pentoxide to orthophosphate. The solution was passed through a 10cm long resin column, and the filtrate was collected. In a 10 mL pyrex test tube. Two milliliters

of the colour development reagent was added and the absorbance was taken at 650 nm.

Determination of Anti-Nutritional Factors

Determination of heavy metals

Five grammes of each sample was weighed out into a platinum foil and dried. The dried samples and the ashes produced were digested with 20 mL of 1.1 (v/v) HNO₃ and HCL acids in 100 mL beaker. The digests were filtered and the filtrates diluted using de-ionized water to 100 mL. Thereafter, 2 mL aliquots was used for the heavy metals determination with the aid of flame atomic absorption spectrophotometer (Buck Scientific, model 210 VGP, USA) using aqueous calibration standards from standard stock standard for each elements.

Estimation of phytic acid

The phytic acid content of the sample were determined by a colorimetric method as previously described (Vaintraub and Lapteva, 1988). A suitable aliquot was diluted with distilled water to make 3 mL and was used for the assay. The results were expressed as mg/g of dry matter using standard phytic acid.

Determination of oxalate

Oxalate content was determined using a previously reported method (Ijarotimi, 2017). One gram of the sample was weighed into 100 ml conical flask, then, 75 mL of H₂SO₄ (3 M) was added. The solution was stirred carefully periodically with a magnetic stirrer for 1 h and was then filtered with Whatman No 1 filter paper. Thereafter, 25 mL of the filtrate titrated hot (80 - 90 °C) against 0.1 M KMnO₄ solution until a faint pink colour persisted for at least 30 s.

$$\text{Oxalate} \left(\frac{\text{mg}}{\text{g}} \right) = V_T \times 0.9004$$

Where V_T = titre volume (mL)

Determination of trypsin inhibitor

The analysis of trypsin inhibitor was carried out according to previous method method. One gram of the sample was weighed into a beaker containing a magnetic stirring bar, before 50 mL of sodium hydroxide solution were added and the suspension was agitated slowly. After 3 h, the pH was measured; pH ranged between 8.4 and 10.0. An aliquot of suspension was taken with a serological 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 was 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. Two milliliters (2 mL) trypsin solution was added to each tube and quickly mixed on the vortex stirrer and placed in the water bath (37°C). Five milliliters (5 mL) BAPNA was added to each tube, mixed on vortex stirrer. The samples were incubated for 10 min at 37°C. After exactly 10 min, the reaction was stopped by addition of 1 mL acetic acid solution followed by mixing on the vortex stirrer. A blank sample 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. A unit of trypsin is considered to be the quantity of enzyme, which will produce 0.01 units after 10 minutes of reaction for each 10 mL of reaction volume at an absorbance of 410 nm by. Trypsin inhibitor activity is defined as the number of trypsin units inhibited (TIU). Absorbance of blank – absorbance of sample TIU (/mL) = 0.01 × volume of diluted sample solution, mL TIU is plotted against the volume of the diluted sample solution.

Determination of hydrogen cyanide

Determination of residual cyanide: Thirty grammes (30 g) of milled “Lafun” sample was homogenized with 250 mL of 0.1 M orthophosphoric acid. The homogenate was centrifuged and the supernatant were taken as the extract; 0.1 mL of the enzyme were added into 0.6 mL of the extract. 3.4 mL of acetate buffer (pH 4.5) was also added,

stirred and mixed, after which 0.2 mL of 0.5% chloramin-T and 0.6 mL of colour reagent were added and allowed to stand for 15 min. for colour development. The absorbance value was obtained at 605 nm wavelength against a blank similar preparation containing all reagents and 0.1 mL phosphate buffer instead of KCN. The data from the standard was used to obtain a standard curve and its slope (b) by plotting absorbance values (Y-axis) against standard concentration (X-axis). The unknown mean absorbance (A) and the weight of the sample (W) were used to calculate the residual cyanide (RS), using the formula:

$$RS = A \times 250 \times 0.4151 \times b \times w$$

The result was expressed as mg HCN equivalent kg⁻¹.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and the means were separated using Duncan Multiple Range Test at (P≤0.05) using Statistical Package for Social Scientists (SPSS) version 17.

Results and Discussion

Amino Acid Profile of “Lafun” Samples

The amino acid composition and nutritional quality of “Lafun” samples are presented in Tables 1 and 2, respectively. The total non-essential amino acids (TNEAA) composition of the control and enriched samples ranged from 33.0 (CS) to 39.50 g/100 g protein (LER) with glutamic acid being the most abundant non-essential amino acids for all the samples. Glutamic acid is often the most abundant and dominant amino acid in food proteins of plant origin (Aderinola et al., 2018; Deng et al., 2015; Olagunju et al., 2018; Wang et al., 2008). Aspartic and glutamic acid are nitrogen reserves in plant where they are stored as ammonia. Regarding the contents of essential amino acids in the samples, all the samples showed considerable contents of the essential amino acids when compared to the WHO reference value. However, enrichment with soy curd or residue also increased the contents of the amino acids, especially, the contents of the essential amino acids. The concentration of total essential amino acids (TEAA) ranged from 27.91 (CS) to 42.78 g/100 g (LEC). Lysine and leucine were the essential amino in higher concentration with 5.22 and 4.36 g/100 g protein, respectively while the concentration of methionine was the lowest. While enrichment with residue marginally increased methionine content above the reference value (2.2 g/100 g) to 2.60 g/100 g, enrichment with the curd on the other hand increased the methionine content to 9.92 g/100 g protein. The apparently significant increase may be due to the higher content of methionine in soy beans curd.

The nutritional quality of control and enriched samples (Table 2) shows that the percentage of essential amino acids (EAAs) in “Lafun” sample enriched with soy curd (42.78%) was higher and significantly different (P≤0.05) from other samples e.g. (LER: 34.47%), (CL: 28.19%) and (CS: 27.91%). In comparison with the contents of essential amino acids from other plant sources, the values obtained in this study for the control (CL) and commercial (CS) samples are significantly lower (Gindy, 2018; Steve and Olufemi, 2019). While the residue supplemented “Lafun”

was moderate, only the curd supplemented “Lafun” contained significant contents of essential amino acid (42.78%). Protein efficiency ratio (PER) and the biological value (BV) were two indices used in the study to evaluate the protein quality of the samples. While these indices, especially the BV revealed poor protein quality, enrichment with curd and residue significantly improved the protein quality parameters. The curd and residue had 223 and 168% increase in BV while for the PER, an increase of 241 and 151% obtained for the curd and residue when compared to the control sample (CL). Nutritionally, the calculated protein efficiency ratios (PER) of the sample

enriched with soy residue (1.12) and curd (1.78) were comparable to cowpea (1.21), millet (1.62) and pigeon pea (1.82) but less than that reported for fermented African locust bean, casein (2.5) (Oyarekua and Eleyinmi, 2004). Higher protein quality indices in the enriched samples may be due to the higher protein contents in them as revealed by their higher total amino acids (TAA) contents. Previous studies also reported increase in protein/amino acid contents in foods enriched with soybeans (Sanni and Sobamiwa, 1994; Waliszewski et al., 2002; Odunayo et al., 2017; Adeyeye et al., 2017).

Table 1. Amino acid composition of “Lafun” samples (g/100 g protein)

Amino acids	CL	CS	LER	LEC	Ref*
Non-essential amino acids					
Alanine	2.56	2.06	3.74	3.68	
Aspartic	5.48	5.4	6.46	5.97	
Serine	2.55	2.59	3.13	3.15	
Glutamic	15.18	14.91	16.26	16.31	
Proline	2.44	2.58	3.30	3.04	
Glycine	2.02	2.01	2.39	2.54	
Arginine	2.55	2.42	3.13	3.15	
Tyrosine	1.74	1.59	2.31	1.95	
Cysteine	2.03	2.00	1.91	1.89	
Essential amino acids					
Phenylalanine	2.04	2.00	2.83	3.08	
Methionine	1.05	1.11	2.60	9.92	
Lysine	5.22	5.42	5.47	5.86	5.2
Threonine	3.40	3.26	2.55	3.02	2.7
Valine	2.69	2.58	3.66	3.74	4.2
Isoleucine	2.78	2.59	3.66	3.41	3.1
Leucine	4.36	4.38	6.84	6.39	6.3
Histidine	2.49	2.48	2.16	2.35	1.8
Tryptophan	1.61	1.67	1.57	1.86	0.7
TArAA (Pheny+Tyro)	3.78	3.59	5.14	5.03	4.6
TSAA[Meth+Cys]	3.08	3.11	4.51	11.81	2.6

KEY: CL = Control sample, CS= commercial sample LEC, = “Lafun” enriched with 10% curd, LER= “Lafun” enriched with 10% residue, Ref* [21] for children aged 1-2 years, TArAA = total aromatic amino acids, TSAA = total sulphur amino acids

Table 2. Summary of calculated nutritional quality of formulated “Lafun” food samples

Parameters	CL	CS	LER	LEC
TAA[mg/100g]	62.19	61.14	73.97	81.31
TEAA	28.19	27.91	34.47	42.78
TNEAA	34	33.23	39.50	38.53
%TEAA	45.33	45.65	46.60	52.61
%TNEAA	54.67	54.35	53.40	47.39
BV[%]	22.97	22.86	38.66	51.26
PER	0.74	0.78	1.12	1.78

KEY: CL = Control sample, CS= commercial sample LEC, = “Lafun” enriched with 10% curd, LER= “Lafun” enriched with 10% residue, TAA = total amino acids, TEAA = total essential amino acids, TNEAA = total non-essential amino acids, BV = biological value, PER = protein efficiency ratio

Mineral Composition of “Lafun” Sample

Mineral elements are essential micro-nutrients required by the body in various quantity for proper functioning and regulation of the body (Aderinola 2018; Krupodorova and Sevindik, 2020; Saridogan et al., 2021). While nutrients such as Na, Ca, K, Mg, and P are required at higher concentrations, Fe, Zn, Mn, Cu are generally required at lower concentrations and are therefore referred to as trace element. On the other hand, the presence of elements such as Pb and Cd are generally not desired. These so called heavy metal are indication of contaminations and their

presence at higher concentrations may be injurious to health. Considering the fact the traditional processing methods for cassava and its products including “Lafun” often predispose the product to heavy metal contamination, their presence was evaluated in this study. Table 3 shows the effect of the enrichment on the mineral content of the enriched “Lafun” samples. Generally, enrichment with curd or residue led to increased mineral contents in the enriched samples except for Na (LER) and K (LEC) where the contents of these minerals were lower when compared to the value obtained for other non-enriched samples. It

was also observed that enrichment with residue significantly increased the mineral contents compared to enrichment with the curd except for Na. All the samples showed very high contents of K when compared to the contents of other major macro nutrients determined in this study. This may indicate the relative abundance of the mineral in cassava tuber. Previous study (Isaac-bamgboye et al., 2020) also confirmed higher contents of K in a cassava product (“Pupuru”) when compared to other elements determined; however, the concentration of K in the study on “Pupuru” for the control sample (597 mg/ 100 g) is significantly higher than the concentration obtained for the control sample (205 mg/100 g) in the current study. Furthermore, while the addition of the curd showed no significant difference between the potassium content of the curd-enriched sample (LEC) and the control sample (CL), the addition of the residue significantly led to more than 300% increase. An earlier study on soy-enriched tapioca (Samuel et al., 2012) also reported a significantly progressive increase in potassium contents of the enriched samples as the level of supplementation with soybeans increased. Potassium is an essential components of body cells and fluids. It is vital and needed for the regulation of heart rate and controlling of blood pressure (Aderinola and Abaire, 2019). The significantly higher Ca/P ratio of the samples, which also increased with the supplementation with curd and residue, than the recommended minimum of 1 may indicate the potential of the products especially,

samples supplemented with the curd and residue to provide adequate supply and utilization of calcium and phosphorus for proper bone formation (Ijarotimi and Keshinro, 2012; Isaac-bamgboye et al., 2020). The enriched samples are better sources of minerals compared to the non-enriched samples. This agrees with previous findings that soy-bean is a rich source of micronutrients such as minerals (Samuel et al., 2012; Odunayo et al., 2017). Higher concentration of potassium in relation to sodium content is essential preventing blood pressure and other cardiovascular risks. 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 (Samuel et al., 2012). High concentration of heavy metals could be attributed to heavy vehicular traffic, or pollution from pesticides, irrigation water and the soil used for planting (Elbagermi et al., 2012). Exposure to high concentration of Pb has been implicated in kidney disease and consequent renal failure. Furthermore, excessive accumulation of heavy metals such as Cd and Pb may cause serious health problems. The non-detection of the presence of heavy metals (Pb and Cd) may indicate that the products were hygienically produced and were not contaminated with these metal. Apparently, these values are lower compared to the maximum concentration level recommended by WHO/FAO of 0.3 and 0.2 mg/kg for heavy metals - Pb and Cd, respectively (Husain et al., 1995). Therefore, the products may be considered safe for consumers.

Table 3. Effect of enrichment on the mineral content of “Lafun” samples (mg/100 g)

Sample	CS	CL	LEC	LER
Ca	62.53±0.04 ^c	60.64±0.01 ^c	103.89±0.25 ^b	111.30±0.84 ^a
Mg	19.61±0.13 ^c	19.62±0.07 ^c	74.64±0.28 ^b	86.51±0.32 ^a
Na	14.29±0.01 ^a	15.20±0.00 ^a	15.38±0.01 ^a	11.21±0.01 ^b
K	212.08±0.56 ^b	204.56±0.05 ^c	203.81±0.85 ^c	644.17±0.01 ^a
Fe	0.29±0.00 ^c	0.27±0.01 ^c	1.37±0.01 ^b	3.21±0.02 ^a
Zn	0.32±0.00 ^c	0.28±0.00 ^c	0.68±0.01 ^b	1.43±0.00 ^a
Mn	0.38±0.00 ^c	0.38±0.01 ^c	0.53±0.00 ^b	1.15±0.01 ^a
Cu	0.12±0.00 ^c	0.13±0.01 ^c	0.22±0.01 ^b	0.56±0.00 ^a
P	20.27±0.07 ^c	26.24±0.71 ^b	26.39±1.25 ^b	31.42±0.01 ^a
Pb	0.00±0.01 ^a	0.00±0.03 ^a	0.00±0.02 ^a	0.00±0.01 ^a
Cd	0.00±0.02 ^a	0.00±0.03 ^a	0.00±0.05 ^a	0.00±0.03 ^a
Ca/P	2.08 ^c	2.30 ^c	4.44 ^a	3.54 ^b
Na/K	0.06 ^{ab}	0.04 ^{bc}	0.08 ^a	0.02 ^{cd}

Values are means of triplicate readings ± standard deviation. Values with different superscripts across rows are significantly different (P<0.05). KEY: CL = Control sample, CS= commercial sample LEC, = “Lafun” enriched with 10% curd, LER= “Lafun” enriched with 10% residue.

Anti-nutritional Composition of “Lafun” Samples

Tannins are anti-nutritional factors; they are water-soluble polyphenols in many foods of plant origin (Khasnabis et al., 2015). It is reported to have astringent taste which often affects palatability. The ability of tannins to react with other essential food components such as proteins, polysaccharides and metal ion is of nutritional and physiologically importance (Atanassova and Christova-Bagdassarian, 2009). Tannin can react with proteins, hydrolyse it and thus reduce the digestibility of the protein (Samuel et al., 2012). Condensed tannin contents of “Lafun” samples increased from 0.51 (CS) to 3.04 mg/g (LEC). The enriched samples (LEC and LER) showed some significant differences (P<0.05) among the samples. It could be inferred that enrichment of “Lafun” with soy supplement is responsible for the observed

increase in tannin contents of LEC and LER. The low tannin content of the “Lafun” samples could be responsible for the absence of bitter taste in the “Lafun” samples. While the reduction of tannins in the dried “Lafun” samples may be due to tannins being thermally labile and sensitive to oxidation. Another study on the supplementation of a cassava product (*gari*) with soy beans also showed increased contents of tannins with increased level of supplementation (Samuel et al., 2012). However, the tannin contents in the study were significantly lower than those reported in the current study.

Phytate, a compound of inositol, is the storage form of mineral phosphorus in plants. They are also considered as anti-nutrients due to the fact that they bind iron and calcium preventing their proper absorption (Me and Aw, 2009). The result of phytate content of “Lafun” samples were shown

in Table 4. The phytate contents ranged between 3.73 (CS) to 6.42 mg/g (LER). Significant difference existed between the enriched (LEC, LER) and the control samples (CS and CL). The concentration obtained in the current study are quite higher than those reported in the literature for other plant foods such as 0.2 mg/g for raw *Senna siamea* seeds (Ingweye et al., 2010) 0.59 and 1.49 mg/g for *Moringa* flour and cake, respectively (Abiodun et al., 2012), but considerable lower than those reported for raw and germinated sesame seeds (Olagunju and Ifesan, 2013). Phytates are reported to be associated with proteins in legumes and other seed proteins. It was also observed that a purified or isolated plant protein may contain higher contents of phytate. This observation may account for the increased phytate contents recorded for the enriched samples.

The oxalate value of the “Lafun” samples also showed a significant difference ($P < 0.05$) between the enriched, commercial and control samples. Oxalate content (Table 4) shows that the enriched samples 1.19 (LER) and 1.18 (LEC) were significantly higher than the non-enriched samples 0.14 (CL) and 0.13 (CS). The oxalate values obtained for the control samples were generally comparable to those reported for green, red and yellow cultivars of *Dioscorea bulbifera* flour (Princewill-Ogbonna and Ibeji, 2015). However, those of the enriched samples were significantly higher. Higher oxalate contents than those obtained for the enriched samples had also been reported in the literature for other plant foods such as 4.12 and 5.31 mg/g for *Moringa* flour and cake, respectively (Abiodun et al., 2012). Oxalates have been reported to be of no usefulness to human health. In fact, high

concentration of oxalate in food are associated with irritation of the tissue and the digestive systems and specifically of the kidney and stomach

Hydrogen cyanide has been implicated as a causative agents in some health challenges such as goiter, ataxic neuropathy, cretinism, and xerophthalmia and stunted growth in children (Ndam et al., 2019). Hydrogen cyanide contents of the various samples are shown in Table 4. Although, all the samples were well below the 10 mg/kg maximum cyanide content recommended by the Nigerian Industrial Standard (Sanni et al., 2005), the cyanogenic potential was significantly low in the enriched samples compared to the control “Lafun” samples. Cyanogenic potentials decrease from 4.34 mg/kg (CS) to 2.27 mg/kg (LEC). There was no much significant difference ($p < 0.05$) between the commercial and control samples. The observed lower hydrocyanic acid contents could be due to the effects of fermentation, dilution (with the supplements) and the heating involved during drying. These values are significantly lower than 12 and 20 mg/kg reported for *gari* and cassava flour (Kemdirim et al., 1995) but comparable to the values obtained for soy-enriched tapioca (Samuel et al., 2012). According to a previous report (Burns et al., 2012), the lethal doses of cyanide is a function of body weight and ranged between 5- 35 mg for a 10 kg body weight to 50-350 mg for a 100 kg body weight.

The presence of trypsin inhibitors in foods can also affect protein digestibility by reducing the activity of trypsin enzyme which plays vital role in protein digestibility in the stomach. The values obtained in these study are comparable to those reported for soy enriched *gari* (Samuel et al., 2012).

Table 4. Anti-nutritional contents of “Lafun” samples

Samples	Condensed Tannins (mg/g)	Phytic acid (mg/g)	Oxalate (mg/g)	Hydrogen Cyanide (mg/kg)	Trypsin Inhibitor (mg/g)
CL	0.53± 0.06 ^b	3.77±0.01 ^b	0.14±0.00 ^b	4.07±0.01 ^{ab}	0.09±0.35 ^b
CS	0.51± 0.00 ^b	3.73±0.20 ^{bc}	0.13±0.00 ^b	4.34±1.29 ^a	0.07±0.55 ^b
LER	2.95± 0.01 ^a	6.42±0.00 ^a	1.19±0.00 ^a	2.34±0.02 ^c	2.09±0.33 ^a
LEC	3.04± 0.03 ^a	5.52±0.00 ^a	1.18±0.00 ^a	2.27±0.02 ^c	1.84±0.36 ^b

Values are means of triplicate readings ± standard deviation. Values with different superscripts across same column are significantly different ($P < 0.05$). KEY: CL = Control sample, CS= commercial sample LEC, = “Lafun” enriched with 10% curd, LER= “Lafun” enriched with 10% residue.

Conclusion

Overall, enrichment with soy curd or residue gave a better product with improvement in the amino acid profile and mineral contents. There was a slight increase in anti-nutrients due to the enrichment process but they were within safe levels and are relatively comparable to those reported for other food items of plant origin. Enrichment with soy curd or residue is a potential avenue to improve the nutritional content of “Lafun”.

Declarations

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preparation: [Uche Anyaiwe]; Writing - review and editing: [Taiwo Aderinola], Supervision: [Matthew Oluwamukomi, Tayo Fagbemi]

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