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Assessment of Anti-Nutritional Factors and Amino Acid Profiles in Rice-Africa Yam Bean Flour Blends

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Abstract: This study evaluates the nutritional enhancement potential of rice-African yam bean (AYB) flour blends, focusing on anti-nutritional factors and amino acid profiles. Seven flour blends were created, ranging from 100% broken rice flour (Sample A) to 70% rice and 30% AYB (Sample G). Results indicated that the total amino acid (TAA) content significantly increased with higher AYB substitution, reaching 107.268 g/100g protein in Sample G, compared to 46.546 g/100g protein in the control. However, corresponding increases in anti-nutritional factors were observed, with trypsin inhibitors rising from 0.290 mg/100g in Sample A to 0.810 mg/100g in Sample G, and phytates increasing from 0.976 mg/100g to 3.443 mg/100g. Despite these increases, all anti-nutritional factors remained within acceptable limits for human consumption. The findings suggest that processing methods such as soaking, fermentation, and cooking can mitigate these factors, thereby enhancing nutrient bioavailability. This research highlights that integrating AYB flour into rice can significantly boost protein quality, presenting a sustainable solution to combat dietary deficiencies in regions reliant on rice as a staple food.

1. Introduction

In recent years, there has been growing interest in the potential of combining staple food crops with legumes to improve nutritional quality and address nutrient deficiencies in many developing regions. One such promising combination is the blending of rice and African yam bean (*Sphenostylis stenocarpa*) flour. Rice, a globally recognized staple, is predominantly composed of carbohydrates, providing essential energy but lacking adequate amounts of key nutrients, particularly proteins and amino acids. In contrast, African yam bean, a lesser-known legume, is a rich source of protein, amino acids, and micronutrients, which could complement the nutritional profile of rice. However, African yam bean contains antinutritional factors (ANFs), such as lectins, tannins, and phytates, which can inhibit the absorption of these nutrients and reduce the overall bioavailability of essential nutrients. Understanding how to mitigate these antinutritional compounds while improving the amino acid composition in rice-African yam bean flour blends could lead to more nutritionally balanced and sustainable food products (Baiyeri & Samuel-Baiyeri, 2023; Atinuke, 2015).

The human body needs proteins together with amino acids to perform many vital physiological duties that involve tissue development as well as biochemical process control. The proteins building blocks called amino acids perform vital biological functions that include enzymes' operations and neurotransmitter creation and immune response activation. The human body requires essential amino acids that only come from food sources to trigger growth alongside muscle repair and control complete metabolic activity. Modern diets rely on plant-based proteins for sustainable and nutritious alternatives to animal proteins because of their significant attention in recent years (Kumar *et al*., 2022; Tan *et al*., 2023). Also, the mass industrial sector uses proteins and amino acids as key substances for multiple applications. Amino acids serve in pharmaceuticals to build delivery systems and therapeutic products which advance medical therapy according to Boukhabza *et al*. (2017) & Bongioanni *et al*. (2022). amino acids are also in corrosion protection (Barouni *et al*., 2014; Aouniti *et al*., 2013 & 2017). Egg albumen proteins find use in construction materials to enhance the physical characteristics of lime mortars used for preservation purposes (Mydin, 2018). The extensive range of applications shows proteins and amino acids adapt well to various settings of both medical needs and industrial demand.

Rice, despite being a staple food in many parts of the world, is deficient in lysine, an essential amino acid, making it an incomplete protein source on its own. However, the amino acid profile of rice can be enhanced when combined with legumes like African yam bean, which is rich in lysine, methionine, and other essential amino acids (Liyanaarachchi *et al*., 2021; Farooq *et al*., 2023). The amino acid composition of rice can vary depending on the variety, processing techniques, and environmental factors. Recent studies have indicated that certain rice varieties are richer in essential amino acids compared to others, and the application of various processing methods can further enhance the digestibility and nutritional quality of rice-based products (Yao *et al*., 2023; *Zhang et al*., 2021). For example, broken rice, often considered a byproduct, has been shown to have distinct nutritional and energy profiles that vary depending on its processing technique, which could influence the overall quality of rice-based blends (Gunha *et al*., 2023; Abd Ghani *et al*., 2023).

On the other hand, African yam bean, which is rich in proteins and micronutrients, has been identified as a potential solution to protein deficiencies in regions where rice is a staple. However, its use is limited by the presence of various antinutritional factors, which reduce the bioavailability of essential nutrients. Several studies have reported on the antinutrient composition of African yam bean, noting the presence of compounds such as tannins, phytates, and lectins, which can affect nutrient absorption (Baiyeri & Samuel-Baiyeri, 2023). While these compounds play protective roles in the plant's defense mechanisms, they can negatively impact human nutrition. Processing techniques, such as fermentation, cooking, or extrusion, have been found to significantly reduce the levels of these antinutritional factors and enhance the digestibility of the nutrients in African yam bean (Omojokun *et al*., 2021; Anosike *et al*., 2020). Understanding how to effectively process and combine rice and African yam bean flours to reduce the impact of antinutritional factors while improving nutrient content is crucial for developing functional food products.

The potential for creating nutritionally balanced, protein-enriched food products from rice-Africa yam bean flour blends is an area of great interest. By combining the energy-dense carbohydrate content of rice with the protein-rich profile of African yam bean, these blends could provide a more complete source of nutrition. Recent studies have shown that different rice genotypes exhibit varying amino acid compositions, with some varieties being more favorable for combining with legumes like African yam bean to create balanced nutritional profiles (Liyanaarachchi *et al*., 2021; Anene *et al*., 2023; Farooq *et al*., 2023). In addition, processing methods such as fermentation and extrusion have been shown to significantly impact the amino acid profile and functional properties of rice and legume-based flour blends (Babarinde *et al*., 2019; Omojokun *et al*., 2021). The combination of rice and African yam bean flour presents an innovative approach to enhancing the nutritional value of staple foods. By addressing the challenges associated with antinutritional factors and optimizing amino acid profiles through processing techniques.

2.Methodology

2.1 Sourcing of Experimental Materials

Broken rice (*Oryza sativa L.*), African yam bean (*Sphenostylis stenocarpa*), baker's yeast (*Sacharomyces cerevisiae*), Sodium bicarbonate (kanwa), Sugar and Salt was purchased from a local market in Makurdi, Benue State. The experiment was carried out in the CEFTER food laboratory of the Benue State University, Makurdi. Makurdi is located in North central Nigeria along the Benue River, on latitude 07°43'N and longitude 08°35'E. It is situated within the Benue trough, at the lower Benue valley and found in the Guinea Savannah region.

2.2 Experiments

2.2.1 Preparation of masa control (Broken rice alone)

The masa samples were prepared by the method described in the literature (Nkama, & Malleshi, 1998, Akande, *et al*., 2018) with little modification in the recipe as shown in Figure 1

Broken rice
↓
Cleaning
↓
Soaking (30 min)
↓
Drying (3hrs)
↓
Grinding
↓
Sieving (0.5mm)
↓
Flour ← Addition of yeast, sugar and water
↓
Batter
↓
Fermenting (12 hrs at room temperature overnight)
↓
Addition of sodium bicarbonate and salt
↓
Frying (3-6 mins)
↓
Cooling

Figure 1. Flow Chart for the Production of Rice-Based Masa

2.2.2 Preparation of Masa from Blends of Rice and AYB

Broken rice and African yam beam blends masa were prepared by substituting portions of the rice with African yam bean at different substitution levels. Broken rice was prepared using the method described by (Owoicho, *et al*.,2020). The Broken rice kernel was washed with clean water, oven dried at 45°C (for 15 min), it was then grinded into flour using laboratory grinder and sieved through a 0.5mm size mesh and was packaged in Low density polyethylene bags. The African yam bean was prepared using the method described by (Wu, *et al*., 2024). The African yam bean was pre-soaked in water for 2 hours and boiled for 20 minutes to inactivate trypsin inhibitor activity and reduce the beany flavour (Adeyeye, *et al*., 2020). The boiled African yam bean was de-hulled by abrasion. The de-hulled African yam bean was dried, roasted and then milled into flour to obtain African yam bean flour (Ade-Omowaye, Tucker, & Smetanska, 2015). The African yam bean flour was blended with the rice in different proportions of rice and African yam bean flour in the ratio 100:00, 95:5, 90:10, 85:15, 80:20, 75:25, and 70:30.

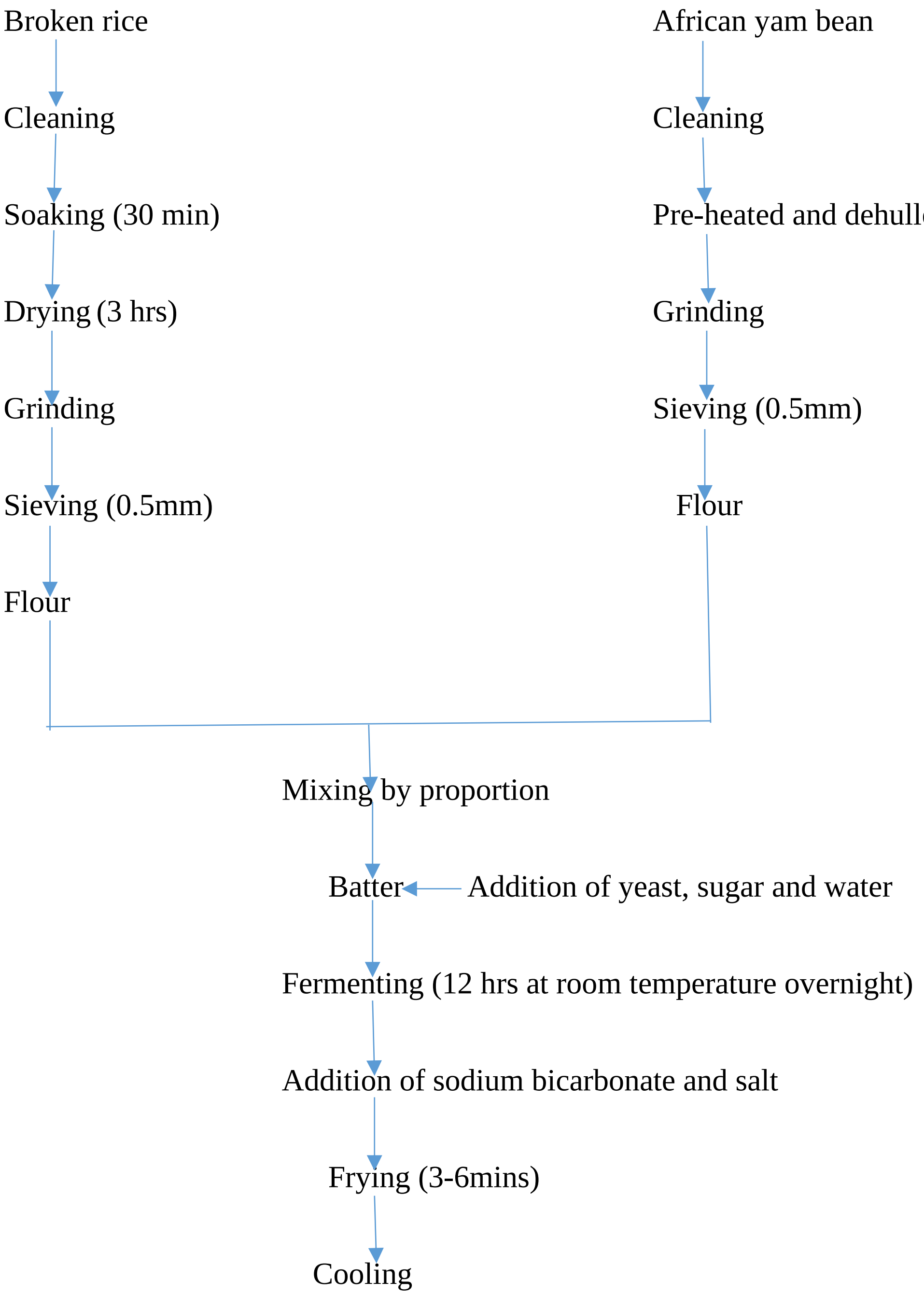

Figure 2. Flow Chart for the Production of Rice-AYB Composite Masa

Table 1. shows different blended formulations of rice flour with African yam bean flour appear within this table. The table displays the use of BRF combined with AYBF at 100% broken rice flour in Sample A and 70% broken rice flour along with 30% African yam bean flour in Sample G. The laboratory design makes it possible to measure changes in nutritional value and anti-nutritional contents as the rice-to-bean concentration changes.

Table 1. Rice and African Yam Bean Blend Formulation

Samples	BRF (%)	AYBF (%)
Sample A	100	0
Sample B	95	5
Sample C	90	10
Sample D	85	15
Sample E	80	20
Sample F	75	25
Sample G	70	30

(key; BRF: Broken rice flour and AYBF: African yam bean flour)

2.2.3 Anti-Nutritional Content Determination

Trypsin inhibitor was determined using the method described by (Liu, 2021). Tannins was determined using the method described by (Hemingway, & Karchesy, 2012). Saponins determination was carried out by (Edewor, *et al.*, 2016) procedure. Oxalate was determined by the method described by (Kasimala, *et al.*, 2018). Phytate was determined by the colourimetric method as decribed by (Gao *et al.*, 2007). The cyanide content was determined using the method described by (Sam, 2019).

2.2.4 Determination of Amino acids

For amino acid analysis, the method described by AOAC (Otter, 2012) was used. Two hundred and fifty (250) mg of samples was hydrolysed with 5ml of 6N HCl (redistilled) at 110°C for 24 hours. The hydrolysate was filtered (Whatman No. 2) into a 50ml flask and lyophilized in a freeze dryer. The dried sample was subsequently dissolved in 3ml of water and again lyophilized. This was repeated three times to remove traces of HCl. The final dried sample was dissolved in about 2ml of water.

An aliquot based on the total amino acids of the sample was taken (5µl of 2.5 mmol), determined as alanine, and dried in a vacuum. The sample was redried using 25µl of ethanol triethyl amine water (2:2:1) and 20 ml of derivatizing solution, ethanol triethylamine water phenylisothiocyanate (7:1:1:1). The sample was incubated at room temperature for 25 minutes, and then excess reagent was removed by drying under vacuum. It was then analysed by reverse-phase high performance liquid chromatography (RP-HPLC) in an amino acid column (Water Associate System, Column Pico Tag) with sodium acetate buffer and acetonitrile. Amino acids were detected at 254nm by analysis with a Shimadzu CR4A Chromatopac. The standard used was Pierce H. amino acid hydrolysate and Amino acids were calculated from the peak areas.

3. Results and Discussion

3.1 Results

Table 2. presents the anti-nutritional factors in different rice-African yam bean masa samples are described in this table. Checkpoints such as trypsin inhibitors and tannins alongside saponins, oxalates, phytates and cyanides exist per 100 grams (mg/100g) and per kilogram (mg/kg) measurements in the analyzed samples at different levels. A systematic evaluation of the anti-nutritional substances occurs as the African yam bean flour content rises (Sample A to Sample G). A statistical analysis (LSD and Duncan's test) shows significant variations between the samples in the provided table.

Table 2. Anti-Nutritional Content of Rice-African Yam Bean Masa

Samples	Trypsin inhibitors (mg/100g)	Tannins (mg/100g)	Saponins (mg/100g)	Oxalates (mg/100g)	Phytates (mg/100g)	Cyanides (mg/kg)
A	0.290 ± 0.010^{a}	0.733 ± 0.005^{e}	0.296 ± 0.015^{a}	0.150 ± 0.010^{a}	0.976 ± 0.015^{a}	0.0012 ± 0.000^{a}
B	0.336 ± 0.015^{b}	0.716 ± 0.005^{e}	0.360 ± 0.010^{b}	0.223 ± 0.005^{b}	1.220 ± 0.010^{a}	0.0016 ± 0.000^{b}
C	0.396 ± 0.015^{c}	0.650 ± 0.026^{d}	0.430 ± 0.010^{c}	0.303 ± 0.005^{c}	1.883 ± 0.005^{b}	0.0022 ± 0.000^{c}
D	0.523 ± 0.011^{d}	0.536 ± 0.005^{c}	0.556 ± 0.005^{d}	0.386 ± 0.005^{d}	2.116 ± 0.005^{b}	0.0025 ± 0.000^{d}
E	0.620 ± 0.010^{e}	0.503 ± 0.005^{b}	0.620 ± 0.010^{e}	0.473 ± 0.005^{e}	2.746 ± 0.015^{c}	0.0034 ± 0.000^{e}
F	0.733 ± 0.005^{f}	0.486 ± 0.005^{b}	0.746 ± 0.005^{f}	0.530 ± 0.010^{f}	3.043 ± 0.005^{c}	0.0036 ± 0.000^{f}
G	0.810 ± 0.010^{g}	0.353 ± 0.005^{a}	0.780 ± 0.010^{g}	0.660 ± 0.010^{g}	3.443 ± 0.574^{d}	0.0037 ± 0.000^{f}
LSD	0.001	0.003	0.001	0.001	0.192	0.098

Values are Mean ± Standard deviation (Values with same superscript on column are not statistically significant (Duncan Multiple range test) at $p \leq 0.05$)

(A= 100% broken rice flour, B= 95:5% rice and African yam bean, C= 90:10% rice and African yam bean, D= 85:15% rice and African yam bean, E= 80:20% rice and African yam bean, F= 75:25% rice and African yam bean and G= 70:30% rice and African yam bean)

Table 3. displays the amino acid examination of rice-African yam bean masa through its essential and non-essential amino acids' milligrams per 100g protein (mg/100g protein) concentration. Testing and statistics show that the table presents amino acid values including tryptophan, histidine, leucine, lysine, and methionine for each rice-African yam bean blend from Samples A to G. The table shows how TAA and TEAA values alter across different samples as African yam bean flour amount grows in the formulated blend.

Table 3. Amino acid Profile of Rice-African Yam Bean Masa (mg/100g Protein)

Amino acid	A	B	C	D	E	F	G	LSD
Tryptophan	1.053 ± 0.005^{a}	1.546 ± 0.015^{b}	2.626 ± 0.015^{c}	3.160 ± 0.010^{d}	4.176 ± 0.015^{e}	4.233 ± 0.015^{f}	4.360 ± 0.010^{g}	0.001
Histidine	0.796 ± 0.015^{a}	0.856 ± 0.005^{b}	1.130 ± 0.010^{c}	1.230 ± 0.010^{d}	1.320 ± 0.010^{e}	1.473 ± 0.005^{f}	1.540 ± 0.010^{g}	0.001

Leucine	1.553± 0.005^{a}	1.746± 0.005^{b}	2.256± 0.011^{c}	3.410± 0.010^{d}	3.653± 0.005^{e}	4.653± 0.005^{f}	4.753± 0.015^{g}	0.001
Isoleucine	1.650± 0.000^{a}	2.010± 0.010^{b}	2.846± 0.005^{c}	2.973± 0.011^{d}	4.040± 0.010^{e}	4.043± 0.011^{e}	5.566± 0.015^{f}	0.696
Phenylalanine	5.886± 0.005^{a}	6.246± 0.011^{b}	7.336± 0.011^{c}	8.136± 0.015^{d}	8.930± 0.000^{ef}	8.940± 0.010^{f}	8.920± 0.010^{e}	0.251
Valine	2.720± 0.010^{a}	2.750± 0.000^{a}	3.846± 0.015^{b}	4.373± 0.058^{d}	3.873± 0.011^{b}	4.170± 0.010^{c}	4.640± 0.010^{e}	0.199
Lysine	3.850± 0.000^{a}	3.863± 0.015^{a}	4.010± 0.010^{b}	4.170± 0.010^{c}	4.260± 0.010^{d}	4.660± 0.010^{e}	5.043± 0.011^{f}	0.141
Methionine	2.656± 0.005^{a}	2.420± 0.010^{a}	2.176± 0.011^{b}	2.090± 0.010^{c}	1.866± 0.015^{d}	1.556± 0.011^{e}	1.220± 0.355^{f}	0.574
Threonine	1.510± 0.010^{a}	1.543± 0.020^{b}	1.870± 0.010^{c}	2.450± 0.000^{d}	3.853± 0.005^{e}	3.936± 0.015^{f}	4.160± 0.010^{g}	0.004
TEAA	***21.674***	***22.980***	***28.096***	***31.992***	***35.971***	***37.664***	***40.202***	
Asparagine	3.603± 0.005^{ab}	3.283± 0.574^{a}	4.233± 0.005^{b}	4.963± 0.011^{bc}	6.323± 0.011^{d}	6.516± 0.015^{d}	6.006± 1.148^{d}	0.633
Arginine	2.710± 0.010^{a}	2.800± 0.010^{b}	3.130± 0.010^{c}	3.780± 0.010^{e}	3.450± 0.000^{d}	3.806± 0.005^{f}	3.980± 0.010^{g}	0.002
Alanine	0.543± 0.011^{a}	0.566± 0.015^{b}	0.700± 0.000^{c}	0.976± 0.015^{d}	0.980± 0.010^{d}	1.380± 0.010^{e}	1.653± 0.005^{f}	0.714
Aspartate	3.213± 0.005^{a}	3.263± 0.058^{b}	4.626± 0.015^{c}	5.643± 0.005^{d}	5.820± 0.010^{e}	5.916± 0.015^{f}	5.916± 0.011^{f}	0.025
Glutamate	1.450± 0.000^{a}	1.770± 0.010^{b}	2.720± 0.010^{c}	3.710± 0.010^{d}	4.153± 0.005^{e}	4.150± 0.000^{e}	4.363± 0.011^{f}	0.625
Glycine	3.650± 0.000^{a}	4.566± 0.011^{b}	6.523± 0.005^{c}	7.133± 0.015^{d}	7.346± 0.005^{e}	8.353± 0.005^{f}	8.626± 0.005^{g}	0.001
Tyrosine	4.103± 0.005^{d}	4.430± 0.010^{c}	3.136± 0.005^{a}	3.466± 0.005^{a}	4.110± 0.010^{b}	11.110± 0.010^{e}	12.180± 0.010^{f}	0.350
Cysteine	3.000± 0.000^{g}	2.726± 0.011^{f}	2.423± 0.015^{e}	2.113± 0.005^{d}	1.946± 0.011^{c}	1.656± 0.005^{b}	1.423± 0.005^{g}	0.001
Proline	1.703± 0.005^{a}	1.710± 0.010^{a}	1.836± 0.015^{b}	2.266± 0.005^{c}	2.286± 0.005^{d}	3.303± 0.005^{e}	5.656± 0.011^{f}	0.393
Serine	2.600± 0.000^{a}	2.746± 0.011^{b}	3.120± 0.010^{c}	3.240± 0.017^{d}	4.843± 0.011^{e}	4.923± 0.005^{f}	6.363± 0.011^{g}	0.001
TAA	***46.546***	***51.174***	***62.143***	***71.302***	***80.628***	***93.077***	***101.568***	

Values are Mean ± Standard deviation (Values with same superscript across row are not statistically significant (Duncan Multiple range test) at $p \leq 0.05$)

(TEA= Total essential amino acid, TAA= Total amino acid, A= 100% broken rice flour, B= 95:5% rice and African yam bean, C= 90:10% rice and African yam bean, D= 85:15% rice and African yam bean, E= 80:20% rice and African yam bean, F= 75:25% rice and African yam bean and G= 70:30% rice and African yam bean).

3.2 Discussion

The inclusion of African yam bean (AYB) flour in rice flour blends has been shown to significantly influence both the amino acid profile and the anti-nutritional factors of the resulting masa. This study demonstrated that varying the proportion of AYB flour in the rice flour blends led to considerable changes in amino acid composition, with a noticeable increase in the total amino acid (TAA) content as the level of AYB substitution rose.

3.2.1 Anti-Nutritional Factor

The anti-nutritional factors of the rice-AYB masa blends were significantly influenced by the proportion of AYB flour incorporated. As shown in Table 2, trypsin inhibitors, tannins, saponins, oxalates, phytates, and cyanides were present in varying amounts across the samples. The levels of these anti-nutrients increased with higher levels of AYB flour substitution, with the highest values found in sample G (70% rice and 30% AYB). This is consistent with previous studies that reported that AYB contains notable quantities of anti-nutritional factors (Ndidi *et al*., 2014; Nwosu, 2013).

Trypsin inhibitors, which are known to interfere with protein digestion, increased from 0.290mg/100g in the control (sample A) to 0.810mg/100g in sample G. Similarly, saponin content also increased progressively, reaching a peak of 0.780mg/100g in sample G. Tannins and oxalates showed a similar trend, with higher levels in the blends compared to the control. Phytates, known for their ability to bind minerals and reduce bioavailability, ranged from 0.976mg/100g in the control to 3.443mg/100g in the highest AYB blend (sample G). These findings suggest that while the inclusion of AYB flour enhances the protein and amino acid content of rice flour, it also increases the levels of anti-nutritional factors, which may impact the bioavailability of nutrients.

However, the levels of anti-nutritional factors in all samples were within generally accepted limits for human consumption, and appropriate processing methods like soaking, fermentation, and cooking could help mitigate their effects. These results are consistent with those of Sam (2019), who found that processing methods significantly reduce anti-nutritional factors in AYB seeds.

3.2.2 Amino Acid Profile

The TAA of the rice flour control sample (46.546g/100g protein) was notably lower compared to the blended samples, which ranged from 51.174g/100g to 101.568g/100g protein. The highest TAA content was observed in the sample containing 70% rice and 30% AYB flour (sample G), which had a TAA value of 107.268g/100g protein. This progressive increase in TAA with the increasing proportion of AYB flour is consistent with previous studies, such as those by Okoye (2015), who reported a similar trend in cassava and AYB blends. AYB, being a leguminous seed, is rich in essential amino acids (EAAs) like phenylalanine, leucine, isoleucine, and lysine, which are often limiting in cereal-based diets. The addition of AYB flour, therefore, enhances the amino acid profile of rice, improving its overall protein quality.

The total essential amino acid (TEAA) content also showed a progressive increase, with the blended samples ranging from 22.980 to 40.202g/100g protein, compared to 21.674g/100g protein in the control. Sample G again showed the highest value of 40.202g/100g protein. These results align with those of Chinonyerem *et al.,* (2017), who noted that leguminous seeds like AYB are excellent sources of EAAs, particularly in diets where animal proteins are scarce.

Among the EAAs, phenylalanine, lysine, and isoleucine were found to be the predominant amino acids in both the control and blended samples. The values of these amino acids were generally higher in the blended samples, with phenylalanine levels ranging from 5.886g/100g to 8.920g/100g, lysine from 3.850g/100g to 5.043g/100g, and isoleucine from 1.650g/100g to 5.566g/100g. These values exceed or approach the FAO/WHO recommended levels for these amino acids, suggesting that the incorporation of AYB flour enhances the nutritional quality of the rice flour-based masa.

However, histidine, which was the lowest in both the control and blended samples, had values ranging from 0.796g/100g to 1.540g/100g, which were below the FAO/WHO standard of 2.80g/100g protein. This may be attributed to the loss of histidine during the roasting process of AYB (Gbenga-Fabusiwa, 2021). Similarly, methionine levels, which are often limiting in legumes, showed a decreasing trend with higher proportions of AYB flour. The control sample (sample A) had the highest methionine content (2.656g/100g), while sample G had the lowest (1.220g/100g), reinforcing the fact that methionine is a limiting amino acid in leguminous seeds (Chinonyerem *et al*., 2017).

In the case of non-essential amino acids, a significant increase was observed in all the blended samples, except for cysteine, which showed a decreasing trend with higher AYB substitution. This finding is in agreement with Omojokun *et al*., (2021), who also observed an increase in non-essential amino acids in flour blends with higher AYB content. The predominant non-essential amino acids found in the blended samples were tyrosine, glycine, serine, and asparagine. Interestingly, tyrosine and glycine levels exceeded the FAO/WHO recommended standard of 1.10g/100g, which suggests that AYB could be a valuable source of these amino acids in complementary blends.

Conclusion

The incorporation of African yam bean (AYB) flour into rice flour blends significantly enhances the nutritional quality by increasing total amino acid content. While higher AYB levels also raise anti-nutritional factors, these remain within acceptable limits for consumption. Appropriate processing methods can mitigate the effects of these anti-nutrients, improving nutrient bioavailability. This study highlights AYB's potential as a sustainable legume for enriching rice-based diets and addressing nutritional deficiencies. Further research is recommended to optimize processing techniques and analyze long-term health impacts.

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