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Nutritionally Enriched Product from Cassava Flour (*Manihot esculenta Crantz)* Fortified with African Yam Bean "Tempeh" Flour (*Sphenostylis stenocarpa)*

Ukpong, S.E.[1]*; Ukanwa, C.C.[2]; Magnus, O.C.[3]; Okoye, A. C.[4]

[1,2]Department of Microbiology, Faculty of Science, Madonna University, Elele, Rivers State.
[3]Department of Pharmacology and Therapeutics, Faculty of Science, Madonna University, Elele, Rivers State.
[4]Department of Industrial Chemistry, Faculty of Science, Madonna University, Elele, Rivers State.

Corresponding Author: Ukpong, S.E.*

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Abstract: The increase in human population especially in developing countries bring key concerns related to food stability, safety and nourishment across the universe. This resulted to huge efforts to explore protein rich foods as a prerequisite for future foods and investigate resilient processing methods. This study aimed at fortifying cassava flour with high protein African yam bean tempeh flour to produce feed for livestock and humans, with improved nutritional and mineral qualities. Varying proportion of blend was produced from wheat and tempeh flour respectively. The blend with proportion (70% CAF: 30% AYBTF) gave the needed consistency. The blend was subjected to cyanide, proximate, mineral, sensory, microbial analyses and animal feeding trial. Standard commercial feed (Top feed) served as control. The cyanide in the cassava flour was 1.5mg/100g while the enriched cassava flour had a cyanide content of 1.0mg/100g. For sensory evaluation, cassava flour recorded higher scores in visual appearance while the fortified cassava flour recorded higher in aroma, texture and taste. Proximate composition of the flour varied from 2.92-11.80%, 3.89- 4.50%, 7.57- 8.09%, 72.72-80.79%, 3.53 - 3.58%, 1.24 - 1.25% for crude protein, total ash, total fat, total carbohydrate, total fiber and flour moisture respectively. Mineral contents; Iron, Magnesium, Calcium, and Phosphorus ranged from 4.18-13.06%, 2.70-32.17%, 61.04-201.42% and 3.76-30.42% respectively. Microbiological analyses (TVC) showed bacterial count of 1.0x10-3.2x10^{3}cfu/mgl and fungal count of 1.0x10-1.5x10^{3}cfu/ml. pH value was 6.3-6.4. The bacterial species isolated and identified using the biochemical tests includes *Bacillus spp., Pseudomonas spp, Coryne bacterium spp, Staphylococcus aureus, Micrococcus spp, and Lactobacillus spp.* Fungi isolated include *Aspergillus, Mucor spp., Penicillium spp., Geotrichum spp*. The weaning wistar rats fed on fortified cassava flour had normal growth and were able to compare favorably with those fed on the commercial feeds. Therefore fortification of cassava flour with tempeh flour gives values of high nutritional quality and is proposed for use in places where cassava products are consumed and protein intake is not sufficient.

Keywords: *Whole-Wheat Flour, African Yam Bean Tempeh Flour, Nutritional Properties, Biscuits, Physicochemical, Antioxidant Properties.*

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I. INTRODUCTION

Malnutrition expressly connotes low energy content, protein or micronutrients in the food consumed by people (Chinma & Gernah, 2007). The quandary of nutritional imbalance in food persists in Africa, partly because animal protein is high-priced to the mass of persons (Azeke, Fretzdorff, Buening-Pfave, Holzapfel & Betsche, 2005). It is a challenge made more critical, by the rapid rate of population increase in Africa, where most person in a population are currently suffering from nutritional imbalance due to food scarcity and war-induced devastation which has also brought about deficiency in proteins to satisfy a burgeoning human population (Onilude, Sanni, & Ighalo 2004).

Legumes are plants with fruits that split open at maturity. Seeds fit for human consumption from plants in the legume family which include beans, soybean and peanut are important but most times looked down on in typical western diets. Legumes are an affordable nutrient-dense protein base replaced for dietary animal protein (Anderson, Smith & Washnock, 1991). While sources of animal protein are often abundant in saturated fats, legumes have unsaturated fats in small measure and plenteous essential mineral, Vitamin B and lysine (Jideani & Onwubali, 2009).

The word tempeh is an Indonesia word which collectively has to do with a wide range of fermented foods (typically soft – cooked legumes) held together by a thick mycelium of fragrant white *Rhizopus* mold into compact cakes (Ko and Hesseltine, 1979). The most demanded parts of tempeh are its attractive aroma, consistency and certain proximate properties. Fresh tempeh has a limited shelf – life. Irrespective of storage temperature fresh tempeh eventually turns brown, the beans becomes visible due to decline of the fungal mycelium, the material softens and pungent smell arises (Nout and Rombouts, 1990). Raw tempeh contains 19.5% protein, compared with 17.9% for hamburger and 21% for chicken, on average. With its high protein content, tempeh serves as a proper protein complement to starchy staples, and can substitute for meat or fish (Nout and Rombouts, 1990; Golbitz, 2009).

The African Yam Bean (AYB) (*Sphenostylis stenocarpa Hochst ex. A. Rich.*) is an under-valued tropical African tuberous legumes found in Nigeria, Central African Republic, Gabon, Zaire and Ethiopia (Ojewola et al., 2006). It is of special value considering that it has twosome food products (grain and tuber). The high protein content of AYB makes it an important source of protein in the diet of many tropical countries. The tuberous roots have a protein composition of 11–19%, while the seed have a protein composition of 21–29% with 3,270 kcal/kg useable energy. The amino acid content, points that its methionine and lysine levels are equivalent to or better than those of soybean. However, the unwanted properties of AYB are similar to those of soybean which are mainly the beans aroma, possession of anti-nutrient components (Liener, 1989; Buono et al., 1990) and difficult-to-cook problem. Kinsella (1985) determined that there are four major criteria determining the approval of new foods or food ingredients. This includes the dietary value, harmlessness, approval and price. Through proper processing techniques and handling methods, AYB can be a harmlessness food source, a good source of roughage, carbohydrate and minerals (Enwere, 1998).

Cassava *(Manihot esculenta Crantz*) is of the family Euphobiaceae. It is an indigenous plant to tropical America (Abercombie and Hickman, 1990), and is one of the most crucial starchy root tubers of the tropics. In Nigeria, cassava is used as a cheap source of energy for human and animal consumption in the form of “kpokpo garri”. The leaves as well as the tuber peels are used as animal feeds especially to goats (FAO, 1997).

II. MATERIALS AND METHODS

- *Sources of Raw Materials*

The cassava (Manihot esculenta Crantz) strain, used for this work was purchased from Rumuodomaya Market, Port Harcourt, while African Yam Bean (AYB) seeds, used in this study were obtained from Igurita market, all in Rivers State.

- *Production of African Yam Bean Tempeh Flour*

African Yam Bean Tempeh flour was processed using the method described by Njoku et al., (1991). The cleaned seeds were boiled at 100ºC for 45 minutes, after which the water was drained and the seeds were allowed to cool to room temperature (25 – 30ºC). The boiled seeds were transferred into a sterile plastic container with puntured covers and inoculated with 5ml of spore suspension containing 10^4 cfu g^{-1}. The injected seeds were fermented at 35ºC for up to 72 hours. Three hundred and fifty grams of bean seeds of good quality was measured and soaked in 1.5 liters of tap water for 12 hours. The soak-water was drained and the seeds were hand peeled. After fermentation, the African Yam Bean tempeh was oven dried at temperature 170 – 230ºC for 12 hours to reduce the water content and then ground using wooden mortar and pestle and then filtered repeatedly to remove testa using a 75µm mesh filter to produce a desired texture and then preserved in a sterile container.

- *Production of Cassava Flour*

Cassava powder was processed through the method outlined by Oboh and Akindahunsi, (2003). The cassava slices were fermented under laboratory conditions. Five liters of distilled water was collected into a ten liter sterile plastic container with plastic cover. The cassava slices were submerged in the distilled water in the plastic container and allowed to ferment for 72hours. The fermented thin slices of cassava were spread out in stainless steel trays and oven-dried for 8-12 hours. The dried cassava were grounded into flour using a blender and sieved repeatedly using a 1.2mm mesh to obtain a product of desired consistency.

- *Nutritional Improvement Process*

The cassava and African yam bean-tempeh flours were integrated at different ratios: 90:10, 80:20, 70:30, 60:40 and 50:50 respectively.

- *Microbiological Analysis of Fermented Cassava Flour (CAF), Tempeh Flour (TEF) Enriched Cassava and African Yam-Tempeh Flour Sample*

The total viable count bacterial of flour samples was evaluated using the pour plate technique as outlined by Giwa et al., (2012). A gram of individual sample was thoroughly mixed in 9ml of sterile peptone water in a sterile 500ml gas jar cylinder. The mixture was evenly mixed and then allowed to settle. A ten-fold dilution was was carried out to and a colony count of $1x10^{-5}$(cfu/ml) gotten, a mill of the reduced sample was introduced into a sterile 9ml Petri dish. Potato dextrose agar (PDA) was poured into the prepared plates for fungi counts. Nutrient agar (NA) was used proper bacteria enumeration, whereas MacConkey agar (MA) was prepared for the enumeration of enteric pathogens. Lactobacillus were enumerated through the use of MRS base agar, incubation

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was done at 37^0C for 72hours. Duplicate plates were prepared. After incubation colonies were taken note of. The different colonies on each plate were isolated, purified and stored on nutrient agar slants (for bacteria) and Potato Dextrose Agar slants (for fungi) for additional characterization and identifications.

- *Characterization and Identification of Isolated Bacteria and Fungi*

The isolated bacterial obtained were identified based the integration of colonial morphology, motility tests and gram staining, as outlined by described by Cheesbrough (2004). Through these tests, the cultural attributes of the isolates evaluated.

- *Evaluation of the Nutritional Composition of the Cassava and Enriched Flour Samples*

The nutritional composition of the formulated products was evaluated. Moisture, ash, crude fiber, fat, protein and carbohydrate contents, were estimated using the method as outlined by AOAC (2010).

- *Sensory Evaluation*

Sensory evaluation was carried out by using the method as outlined by Akinjayeju (2009). From the various blends integrated above, 100gram of each combination was added into 400ml of boiling water in a cooking pot on a gas cooker; wooden stirrer was used in stirring the rehydrated flour continuously to attain a paste of desired consistency. Sensory evaluation was carried out within ten minutes of preparation of the paste the assessment of paste was based on visible appearance, consistency, taste, smell, crust, shape and general acceptability. The assessment was carried out by a semi trained 10-member panel, using a 9-point Hedonic scale with 1 representing the least score (where 1 = strongly oppose, 2 = had a marked dislike for, 3 = had a moderate liking for, 4 = partially dislike, 5 = did not either like nor dislike, 6 = partially liked, 7 = had a moderate liking, 8 = like very much and 9 = fully liked).

- *pH Determination*

The method as outlined by Aderibigbe & Osegboun (2006) was utilised in determining the pH of the flour samples Ten gram of each flour sample was measured and dissolved in 100ml sterile purified water. The suspension was homogenised thoroughly in food blender. The pH was standardized using freshly prepared pH buffer solutions of pH 4.0 and 7.0. The standardization of the metre rule was performed by dipping the electrode into each of the buffer solutions. The electrode was rinsed with distilled water, cleaned with an absorbent cotton wool and dipped in the solution of the sample contained in a test tube of unknown pH. The pH of the sample was read on the display panel

- *Evaluation of Cyanide Content of Fermented and Unfermented Cassava Tubers*

The toxic content of the fermented and non- fermented cassava tubers was evaluated using the Alkaline Picrate Method as outlined by Okoko (2011) whereas the titratable total acidity was determined using the method outlined by Nwafor *et al.*(2015).

- *Animal Feeding Trials*

The method as described by Egonu and Njoku (2006) was used in carrying out a nutritive evaluation, this was carried out by using the animal assay technique using the weaning litter mate rats of the wistar strains obtained from the department of Human Physiology, University of Port Harcourt. The rats were collected at 21-24 days of age, the weight of rats was recorded, rats were also numbered and housed individually in wooden cages. The rats were weaned to acceptable enriched blend of cassava flour diet in the experimental cages for weeks to acclimatize the animal and so that on commencement of feeding trials the rats were always 28-31 days old weighed 35-40g. Half a dozen rats were used and grouped into three groups of two each. Group 1A had rats fed only with standard commercial feed (Top Feed), this served as the control rats. Rats in group 1B were fed with cassava flour alone while rats in group 1C, were fed with a fortified blend of cassava and tempeh flour in the ratio of 70:30. The feeding process was done for a period of four (4) weeks and the average weekly weight of the rat was recorded.

- *Statistical Analyses*

Mean and standard deviations were calculated to analyze sample attributes; proximate analysis and weight measurement during feeding trials, the data obtained were subjected to analysis using a one-way analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

Plate 1 shows the harvested Tempeh (A), the fermented cassava (esculenta Crantz) for cassava flour production (B), Cassava flour (C), African Yam Bean Tempeh flour (D) Blend of cassava and African yam bean flours at the ratio of 70:30 (E).

- *Sensory Analysis*

The result gotten from the sensory evaluation displayed a significant difference ($p<0.05$) statistically. Enriched product recorded higher values in aroma, taste and overall acceptability (Table 1), while cassava flour recorded higher scores in visual appearance, texture and organoleptic attributes.

- *Chemical Changes (pH and Titratable Acidity)*

Cassava flour pH value dropped from about 6.31 to 4.50 at 24 hours and to 4.0 at 72 hours. For the enriched product, significant decrease in pH took place with the formation of mixture and this was most apparent in fresh enriched product blend. Evaluation of variance of the data showed that fermentation period and nature of raw materials affected their pH.

The Titratable Acidity (TA) increased from 0.04(g lactic acid/100g dry sample) to 0.8(g lactic acid/100g dry sample) for cassava flour samples and the enriched formulation increased from 0.07 to 0.13mg g^{-1} (table 5). Variance evaluation reveals that supplementation with African yam bean tempeh affected the acidity of the medium.

Total hydro-cyanide contents of cassava flour and the enriched product are indicated in table 5

The toxic content of the non-fermented cassava was 9.2, while the total toxic content of the fermented cassava flour was 1.5 mg HCN/100g while the nutritionally formulated enriched product had a relatively low total toxic content (1.0mg HCN/100g) because the cassava was diluted by the African yam bean tempeh.

- *Biochemical and Morphological Characteristics of Bacterial Isolates from Cassava Flour, African Yam-Bean Tempeh and the Enriched -Product*

Table 5 shows the biochemical and morphological characteristics of the bacterial isolates from cassava flour, African yam bean tempeh flour and the enriched product respectively. The characteristics of the isolates showed that different bacteria were isolated from cassava flour and African yam bean tempeh. *Bacillus spp., Staphylococcus spp., Micrococcus Lactobacillus spp.* was isolated from African yam bean tempeh and enriched product, whereas *Pseudomonas and Corynebacterium species* was isolated only from cassava flour sample. Also cassava flour had more gram negative bacteria compared to other samples, while genera of gram positive bacteria isolates were the same in cassava flour, African yam bean flour and enriched product. The bacteria isolated exhibited two basic shapes which include the rod and spherical shapes.

- *Characterization and Identification of Fungal Isolates from Cassava Flour, African Yam-Bean Flour and the Enriched -Product*

Table 2 shows the characterization and identification of the fungal isolates from cassava flour, African yam bean tempeh flour and the enriched product respectively. The characteristics of the isolates showed that different fungi were obtained from the samples. The cultures were incubated for five days with visible growth beginning from the third day.

Penicillium spp. was isolated from African yam bean tempeh while *Aspergillus spp., Mucor spp.* and *Geotrichum spp.* were isolated from cassava flour and enriched samples. *Mucor spp.* was isolated from all samples. *Aspergillus spp.* had irregular colonies. It changed colour on continuous incubation, at first it was white, then turned yellow and finally to green. Branched mycelium terminating into conidia was observed. It was isolated from the three samples.

- *Nutritional Composition*

The ash, moisture and lipid contents of cassava flour and enriched cassava flour (cassava flour fortified with African yam bean tempeh) were equivalent (Table 4.9). Tempeh recorded higher scores (3.48, 1.27, 3.64) as against (3.35, 1.24, 3.56) for cassava flour respectively. The carbohydrates content of cassava flour was substantially ($p>0.05$) higher than that of the enriched sample (82.29 versus 75.84). Similarly, higher lipid (6.59 vs 5.98) was observed in both cassava and enriched flour respectively. In contrast, crude protein content was significantly ($p>0.05$) higher in enriched cassava flour than in cassava flour sample, 9.78 and 2.96, respectively.

- *Percentage Occurrence of Microbial Isolates*

Figure 2 illustrates the percentage occurrence of bacterial isolates. According to the results *Bacillus spp.* and *staphylococcus spp.* had the paramount occurrence in cassava flour while *Staphylococcus spp.* and *Corynebacterium spp.* occurred in cassava flour. *Lactobacillus spp.* and *Micrococcus spp.* had equal percentage in cassava flour while *Pseudomonas spp.* and *Micrococcus spp.* had equal percentage in enriched flour (cassava-African yam bean tempeh). All the fungi isolates occurred in cassava flour except *Penicilium spp.* which occurred in enriched and tempeh flour, figure 1.

- *Animal Feeding Trials*

The experimental animals were fed for a period of 4weeks with the weekly weight of the rats documented (table 6), and the average starting weight of the rats was 40g. A substantial steady increase in weight of rats in the group 1 was noticed, the rats in the control group, fed on commercial feed (top Feed) documented mass of about 58.50g at the fourth week. Group 2 rats, fed only on cassava flour and a steady increase in weight of approximately 50.70g at the fourth week was documented. Similar trend also occurred for rats in group 3, which fed on 70/30 mixture of cassava flour and African yam bean tempeh to about 54.80g at week 4.

Table 1 Sensory Attributes of Cassava Flour and Cassava-African Yam Bean Flour.

Sensory attributes	**Aroma**	**Taste**	**Texture**	**Visual Appearance**	**Acceptability**
Enriched product	7.30 $\pm$0.39	6.50$\pm$ 0.27	6.00$\pm$0.21	5.70$\pm$.30	6.40$\pm$0.22
Cassava flour	5.60 $\pm$0.16	5.70$\pm$0.21	6.70$\pm$0.21	6.30$\pm$0.21	6.0$\pm$0.21

Table 2 Morphology of Fungal Isolates from Cassava Flour and Enriched Product

Macroscopy	**Microscopy**	**Parable organism**
White powdery surface, bluish-green with white border. Reverse is white and non-crack	Separate hyphane with branched conidiosphores that have secondary branches known as metulae	*Penicillium ssp.*
Brown/black powdery surface with a b]velvet whitish surrounding, the reverse is cream and crack colony	Non-septate Non—branched sporagiophores no stalons and rhizoids	*Mucor spp.*
Irregular, at first white, then turn to yellow and finally to green	Branched mycelium, condiophores are enlarged at the tip forming a rounded vesicle.	*Aspergillus spp.*

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White and moist, yeast like and easily picked up, with submerged hyphae at the periphery.	Coarse hyphae with segmented rectangular aethrospores with different sizes and round at their edges	*Geotrichum spp.*

Table 3 Proximate Composition of Fermented Cassava Flour and Enriched Product (Cassava-African Yam Bean Tempeh) (g/100g)

Nutrients	**Moisture**	**Protein**	**Ash**	**Fibre**	**Lipid**	**CHO**
Tempeh Flour	2.22±006	28.73±03	3.47±0.05	3.19±0.5	3.76±006	58.63±0.51
Cassava Flour	1.24±0.14	2.92±0.18	3.89±0.69	3.58±0.02	7.57±0.45	80.79±0.47
Enriched Flour	1.25±0.10	4.50±0.70	3.53±0.47	8.09±0.95	8.09±0.95	72.72±0.42

Table 4 Morphological and Biochemical Characteristics Bacterial of Isolates from Cassava and Enriched Flours

Gram rxn	**Shape**	**Catalase**	**Coagulase**	**Motility**	**Starch hyd.**	**Citrate**	**Urease**	**MR**	**VP**	**Spore and hyphae**	**Dnase**	**Lip**	**Gala**	**Glucose**	**Malt**	**Xylose**	**Lactose**	**Fructose**	**Sucrose**	**Manitol**	**Galactose**	**Dextrose**	**Probable organisms**
+ve	Cocci in cluster	+	+	-	-	+	-	-	+	**A**	+	-	-	**A**	**A**	-	-	**AG**	-	**AG**	**A**	**AG**	*Staphylococcus aaureus*
+ve	Rods	-	-	-	-	-	+	+	-	-	-	-	-	-	**A**	-	**A**	**A**	**A**	-	**AG**	**A**	*Lactobacillus spp*
+ve	Rods	-	-	+	+		-	-	+	±	-	-	-	**AG**	**A**	-	-	**A**	**A**	-	**A**	**A**	*Bacillus spp*
+ve	Rods	+	-	+	+	+	-	-	+	±	+	-	-	**AG**	**A**	**A**	-	**A**	-	-	**AG**	-	*Corynebacterium spp*
+ve	Rods	+	-	-	-	+	-	+	-	±	-	-	-	**AG**	**AG**	**A**	-	**A**	**AG**	-	**A**	**A**	*Pseudomonas spp*
+ve	Cocci in pairs	+	-	-	+	+	+	+	-	-	-	+	+	-	**A**	**A**	-	**A**	-	**A**	**A**	-	*Micrococcus spp*

Key: A/G = Acid/Gas positive, + = Positive, - = Negative, VP = Voges Proskauer, MR = Methyl red,

Table 5 Physicochemical Profile of Fermented Cassava Tubers

Parameters	**Before Fermentation**	**Fermentation Time (Hour)**				
		6	**12**	**24**	**48**	**72**
pH	6.5	6.4	6.2	5.71	ND	3.8
Titratable acidity(%)	0.04	0.4	0.07	0.13	ND	0.15
Hydrogen Cyanide(mg/100mg)	9.8	6.3	5.31	4.50	4.0	2.5

ND = Not determined

Table 6 Average Weekly Weights of Rats in Relation to Feed Composition

	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Group 1	40.0+0.0	45.20+3.80	50.30+2.70	54.30+3.70	58.50+3.50
GROUP 2	40.0+0.0	42.20+0.20	45.70+0.70	48.48+1.01	50.70+0.50
GROUP 3	40.0+0.0	42.70+0.50	48. 20+1.0	51.30+1.10	54.80+1.80

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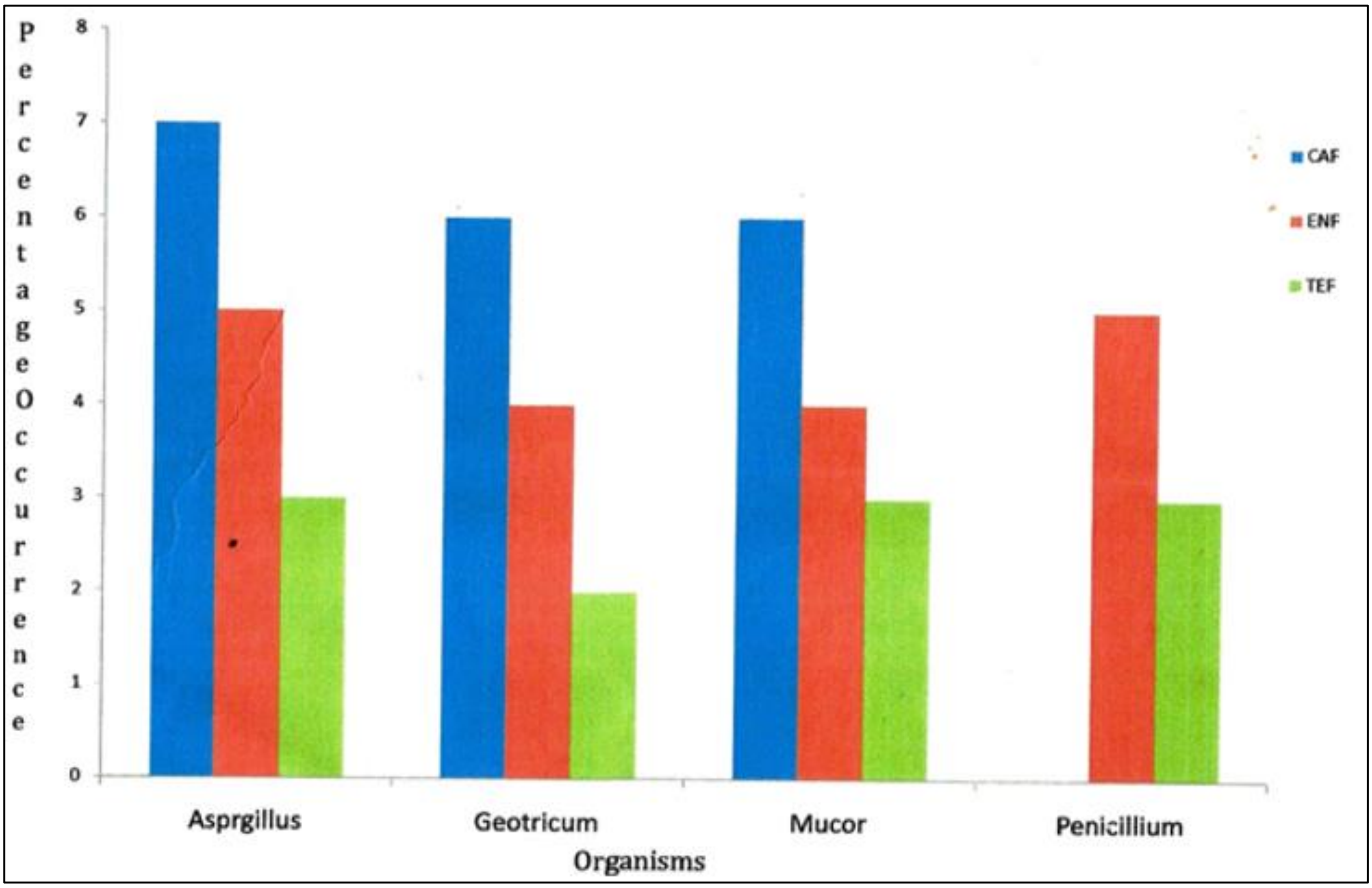

Fig 1 Percentage Occurrence of Fungal Isolates

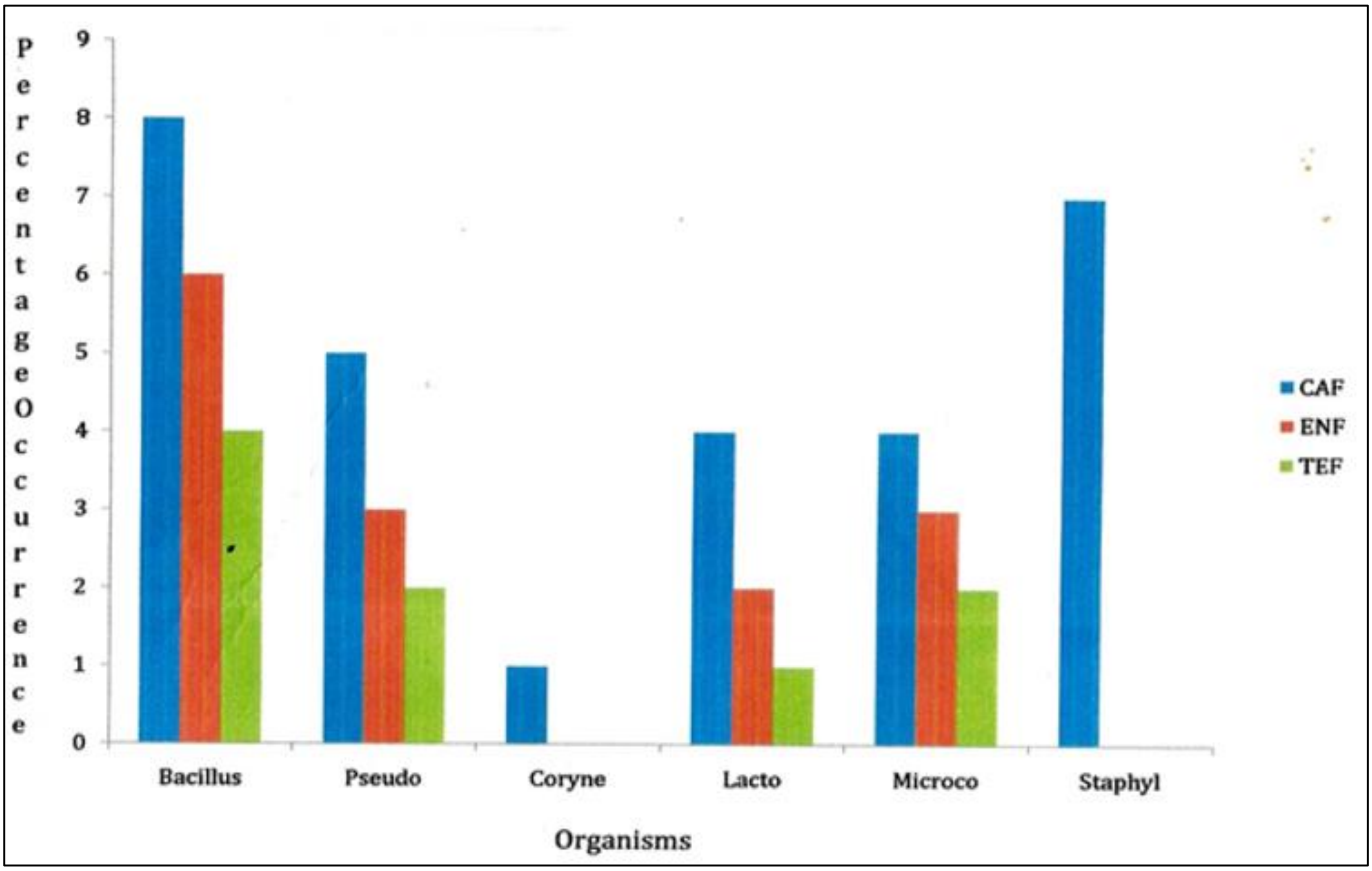

Fig 2 Percentage Occurrence of Bacterial Isolates

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IV. DISCUSSION

The cassava and African yam bean seeds in the course of preparation of cassava and cassava-African yam bean tempeh flours were linked to a broad variety of microorganisms, this result is in agreement with the observations of Aboaba *et al.*, (2022); and Eze and Chukwe (2023).

A comparison of the microbial counts showed different microbial qualities with cassava flour showing higher microbial counts, Perhaps. this may be attributed to the precooking to which the African yam bean seeds underwent fermentation and oven drying after fermentation, the longer fermentation time for cassava (72hours) as against 48hours for African yam bean seeds and the microbial diversity as suggested by Fagbemi and Ijah, (2005) often associated with the soil from which cassava was harvested, water used for fermentation, surrounding air or the hygienic state of the utensils used for the soaking and retting of the tubers, surface micro flora of raw materials and handlers, all have effect on the type of microorganisms isolated during the fermentation process (Abegaz, 2007). Numerous microorganisms are usually involved during fermentation processes but a few types usually determine the end product.

As fermentation progressed with time with the availability of adequate nutrients, microorganisms undergo geometric increase in number (Oyewole and Afolami, 2001). The addition of water to substrate beefs up the growth and enhances the metabolism of the microbial population in the substrate. As the microbial flora increases, the pH alternates and the entire fermentation environment create situations conducive for most organisms such as lactic acid bacteria and yeasts which are capable of tolerating acidic environments and at the same time stops the growth of others organisms such as the enteric organism (Efiuvwevwere and Amadi, 1992).

The drop in pH of cassava from 6.3 I to 4.0 and that of African yam bean tempeh from 6.42 to 5.71 after fermentation was due to the break- down of starch in the substrates by microorganisms with the production of various organic acids such as lactic acid or acetic acid consequently, lowering the pH of the substrates. The presence of both lactic acid bacteria in food (*Lactobacillus spp. and Corynebacterium spp*) is very significant as the acidic P^H inhibits the grow of spoilage microorganisms on the food therefore making the product microbiologically safe.

This results aligns with the observations of Adebayo-Oyetoro et al., (2013). A broad range of aldehyde and esters are responsible for the attractive and admired taste and aroma of the fermented products (Ekwutos, 1998; Ogiehor, 2003; Oboh and Akindahunsi, 2003). Staphylococcus, and Pseudomonas species are of public health importance. When these microorganisms are isolated it flags the possibility of contamination which may come from the methods of handling and Processing utensils and even the environment where the processing took place .The dominance of *Bacillus subtilis* is probably responsible for breakdown of complex compounds during fermentation (Adegoke, 2000; Amusa et al., 2005). The fermentation process, effectively reduced the cyanide potential of the products to safe levels, HCN/100g for cassava flour and 1.0 mg HCN/100g for cassava-African yam bean tempeh, This aligns with the observation of Oyewole, 2001; Osho, 2003 who confirmed through their research works that the fermentation of cassava is an important means of detoxifying it by enzymatic breakdown of linamarin and lotaustralin, there by releasing volatile hydrogen cyanide that escapes.

Studies have shown that there is drastic reduction of microbial population following the preparation of samples with boiled water to form semi solid food which confirms the lethal effect of boiling water as a mode of microbial destruction (Oyeyipo, 2011). Thus, the vegetative cells which could not resist such high temperatures were eliminated. It indeed needful to process cassava products meant for human consumption for proper storability, preservation and palatability but most importantly for for safety. Moreover, the sensory evaluation of the samples revealed that prepared enriched cassava-African yam bean tempeh flour was comparable to cassava flour in visual appearance. Evaluation on physical appearance showed that integration of cassava flour with African yam bean tempeh flour made a significant impact on the color. This may be due to the fact that the African yam bean tempeh had under- gone a drying process during dehydration. The heat application may have made an impact on the colour of food products (Blanca *et al.*, 2009). Prepared cassava flour sample received higher score in visual appearance (6.30). Fermentation and heat treatment may have impacted characteristic aroma of the food which is, retained by food products (FAO, 1997; Oyewole and Afolami, 2001; Blanca et al., 2009). Prepared enriched product (cassava-African yam bean tempeh flour) recorded higher score in aroma (7.30).These results logically follow the observations made in the corresponding aroma attributes. The texture of prepared cassava-African yam bean tempeh flour (6.00) was comparable to prepared cassava flour in this study (6.70) Consumers described good texture as one that does not stick to the hand of the consumers (FAO, 1997; Oboh and Akindahunsi. 2003). However, prepared enriched product scored lower (6.00) than prepared cassava flour (6.70) in texture. Overall, enriched product (cassava-African yam bean tempeh flour) had better quality attributes. From the result of the proximate analysis, Ash, moisture and lipid contents of cassava and cassava-African yam bean tempeh flours were comparable. Cassava-African yam bean tempeh recorded higher scores (4.50, 1.25 and 8.09) as against (3.89, 1.24 and 7.57) for cassava flour, respectively. The carbohydrate content of cassava flour was significantly ($p>0.05$) higher than that of cassava African yam bean tempeh sample (82.29 and 75.84). In contrast, crude protein content was higher in enriched cassava-African yam bean tempeh than in cassava flour sample, (1.78 and 2.92) respectively. The significant increase recorded in protein level is consistent with earlier reports by Efiuvwevwere and Ejikeme (1998), who reported 89.6% increase in total protein content of African pear following inoculation with *Aspergillus niger*. This observation may be partially ascribed to the utilization of products of carbohydrate degradation for protein synthesis by

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the fungus, *Rhizopus oligoşporus* used in the Fermentation of African yam bean seeds and are of nutritional importance especially as cassava is a staple food for rural people where alternative animal proteins are expensive to afford (Obueh, 2017). It has been reported by Ahaotu et al., 2017; Kaale, 2025, the integration of cassava with soybean or cowpea extract brings about a substantial increase in the protein content of cassava.

Furthermore, nutrient availability and substantial nutritive potential of seeds of leguminous plants are observed as some major benefits of fermenting the seeds. In an observation made by Egonu and Nioku, (2006), fermented African oil bean seeds could be incorporated in animal feeds. Studies on vitamin production by three strains of *Rhizopus spp* used in tempeh solid state fermentation outlined that *Rhizopus oligosporus* were generally the best vitamin formers (Aminigo and Obot, 2004). These findings were confirmed in this present work on integration of cassava flour with African yam bean tempeh which gave a highly nutritious and quality feed product comparable to the standard commercial feed (Top feed) used as control during feeding trails. However, there was a significant difference in the final weight of rats fed on cassava flour (50.70g) and integrated enriched cassava-African yam bean tempeh product (54.80g) during the fourth weeks feeding trail.

V. CONCLUSION

Microbial fermentation was found to have significant effect in the reduction of the cyanide contents of both the cassava and African yam bean, increased the protein content and at the same time eliminate pathogens from the fermented products. The data generated gathered from the nutritional analysis and feeding trial experiment pointed out the fact that the Enriched product (cassava-African yam bean tempeh flour) is of substantial nutritive value. Therefore, integration of cassava with African yam bean tempeh results in drastic reduction in malnutrition caused by root crop based Nigerian/ African diets particularly among the financially constrained populations, in addition the enriched product can be explored and used as a weaning food for infants. The procedures which were developed during this research will also help to overcome the issues surrounding the underutilization of African yam bean seeds and convert it into a common and major source protein in Nigeria. This can serve as a guide for making known technologies developed on cassava.

RECOMMENDATION

Based on the findings, the integration of cassava flour with African yam bean tempeh flour significantly increased the protein, ash, fiber, some minerals and improves the amino acid balance, while it lowered the carbohydrate fraction and reduced the cyanide content during fermentation. It is therefore crucial and recommended that cassava, should be fermented before consumption to ensure safety and improved quality.

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