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Production, Sensory and Microbiological Properties of Biscuits Produced from African Yam Bean Tempeh Flour and Wheat Flour

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Abstract:- Foods rich in Carbohydrates but low in protein have been identified as a contributing factor in malnutrition. As a remedy, this study looked into the use of wheat flour fortified with protein-rich African yam bean Tempeh flour, to formulate biscuits with improved nutritional qualities. Six biscuit samples were produced from wheat (Triticum aestivum) and African yam bean seeds (Sphenostylis stenocarpa), respectively. The samples were prepared at different proportions (90:10, 80:20, 70:30, 60:40, 50:50 and 100%) and were subjected to chemical, sensory and microbial evaluations using standard methods of analysis. Sensory parameters evaluated were taste, aroma, shape, colour, crispiness and general acceptability. Biscuits made from composite blends had higher levels of lipids, proteins, crude fiber and crude ash whereas carbohydrate and moisture levels of the biscuits produced from control sample (100% WF) were significantly (P≤0.05) higher than those made from composite flour blends. Biscuit produced from 100% wheat flour had the highest scores for general acceptability and other attributes evaluated. Biscuits produced from 90% WF: 10% AYBTF compared favourably with biscuits produced from 100% WF for almost all the sensory attributes examined. Microbiological analysis (total viable bacterial and fungal count) indicated that all the biscuit samples produced were free of microorganisms for up to three weeks of storage under ambient conditions. This study showed the possibility of producing nutritious and microbiological shelf–stable biscuits from wheat flour fortified with African yam bean Tempeh flour.

Keywords:- *Biscuits, Wheat, African Yam Bean, Tempeh, Microbial, Sensory.*

I. INTRODUCTION

The major increase in snacks intake among people especially in developing countries, may be attributed to socio-economic development. Snacks often contain high calories and fat, but low protein and vitamin content (Rampisad et al., 2003). Blending of wheat with low cost legume flour such as African Yam Bean Tempeh flour in the production of biscuit will help in reducing the problem of protein insufficiency in such developing countries like Nigeria. Biscuits are one of the popular cereal foods, apart from bread, consumed in Nigeria. They are ready-to-eat, convenient and inexpensive food products, containing digestive and dietary principles of vital importance (Okoye & Okaka, 2019). They are nutritive snacks produced from unpalatable dough that is transformed into appetizing product through the application of heat in the oven (Olaoye et al., 2007). Their good eaten quality makes them attractive for protein fortification and nutritional improvements, particularly in children feeding programs, for elderly and low income groups (Banureka and Mahendrn, 2009). Biscuits are prepared with fortified or composite flour to increase their nutritive value (Hasmadi et al., 2014). In Nigeria, ready-to-eat baked products (snacks) consumption is continually growing and there has been increasing reliance on imported wheat (Akpapunam et al., 1999).

Tempeh is an Indonesia word referring collectively to a variety of fermented foods (typically tender – cooked legumes) bound together by a dense mycelium of fragrant white *Rhizopus* mold into compact cakes (Ko and Hesseltine, 1979). The major desirable aspects of tempeh are its attractive flavor, texture and certain nutritional properties. Fresh tempeh has a limited shelf – life. Irrespective of storage temperature fresh tempeh eventually turns brown, the beans becomes visible due to senescence of the fungal mycelium, the material softens and ammoniacal odours emerge (Nout and Rombouts, 1990). Before cooking, 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 tasty 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 one of the under-utilized 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 duo food products (grain and tuber). The high protein composition 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 metabolizable energy. The amino acid composition indicates that its methionine and lysine levels are equal to or better than those of soybean. However, the undesirable properties of AYB are similar to those of soybean which are mainly the beany flavour, possession of anti-nutritional components (Liener, 1989; Buono et al., 1990) and hard-to-cook defect. Kinsella (1985) determined that there are four critical

criteria determining the acceptability of new foods or food ingredients. This includes the nutritional value, safety, acceptability and cost. Through proper processing techniques and handling procedures, AYB products can be a safe food source, which is a good source of fiber, carbohydrate and minerals (Enwere, 1998).

Wheat (*Triticum aestivum*) is a staple food in many parts of the world. It is adaptable to the wide range of environmental conditions (Kent and Evers, 1994). Wheat based foods are a major source of nutrients in many regions of the world. In addition, wheat is also generally used as a source of carbohydrate that generates high glycemic response after ingestion (Ade et al., 2012). It is also a substantive source of protein, vitamins and minerals, when consumed as a major component of diet. It is used extensively in many parts of the world for preparation of different types of breads and many other confectionaries, because of the unique properties of its protein (Gluten) which combines strength and elasticity required to produce bread, cookies, cakes and pastries (Akhtar et al., 2008).

II. MATERIALS AND METHODS

➢ *Sources of Raw Materials*

The African Yam Bean (AYB) seeds, wheat grains and other baking ingredients used in this study were obtained from Mile One market, Rivers state.

➢ *Production of Wheat Flour*

The whole wheat grains were processed using the method described by Enwere (1998). Grains were cleaned from dirt, by sorting out contaminants such as sand, sticks and leaves, thereafter washed and oven dried. The dried whole wheat was milled using attrition mill and sieved into fine flour of uniform particle size, by passing it through a 75µm mesh filter and then stored in an air-tight container.

➢ *Production of African Yam Bean Tempeh Flour*

African Yam Bean Tempeh flour was processed using the method described by Njoku et al., (1991). The dehulled seeds were cooked at 100°C for 45 minutes, after which excess water was drained and the seeds were allowed to cool to room temperature (25 – 30°C). The cooked seeds were transferred into a plastic container with perforated covers and inoculated with 5ml of spore suspension containing 10^4 cfu g^{-1}. The inoculated seeds were fermented at 35°C for up to 48 hours Three hundred and fifty grams of bean seeds of good quality was measured and soaked in 1.5 litres of tap water for 12 hours. The soak-water was drained and the seeds were manually dehulled. After fermentation, the African Yam Bean tempeh was oven dried at temperature 170 – 230°C for 12 hours to reduce the moisture content and then ground using wooden mortar and pestle and then sieved repeatedly to remove testa using a 75µm mesh filter to produce a uniform texture and then stored in an air tight container.

➢ *Blend Formation*

The Wheat Flour and African Yam Bean Tempeh Flour were mixed at various proportions of 90:10, 80:20, 70:30, 60:40, 50:50 and 100:0 %, with 100:0 serving as the control. The measurements were accurately weighed using a standard compact analytical balance, according to the different blend ratios.

➢ *Production of Biscuits*

In the Ihekoronye method of biscuit production, measured quantities of sugar and fat (Margarine) were mixed together in a plastic bowl to a creamy paste. Next, the flour blends, common salt and sodium-bicarbonate were all mixed together in specific ratios as shown in table 1 to produce the dough. The dough was mixed manually for 15 minutes until a uniform smooth paste was obtained. The paste was rolled on a rolling board sprinkled with some flour to a uniform thickness using a wooden hard roller. Circular biscuits were cut (using a circular biscuit cutter of diameter 4cm), placed on a greased baking tray and kept at a normal room temperature for 2 hours to allow proper dough leavening. The biscuits were baked in an oven at a temperature of 184°C for between 15-20 minutes until a very light brown colour was formed.

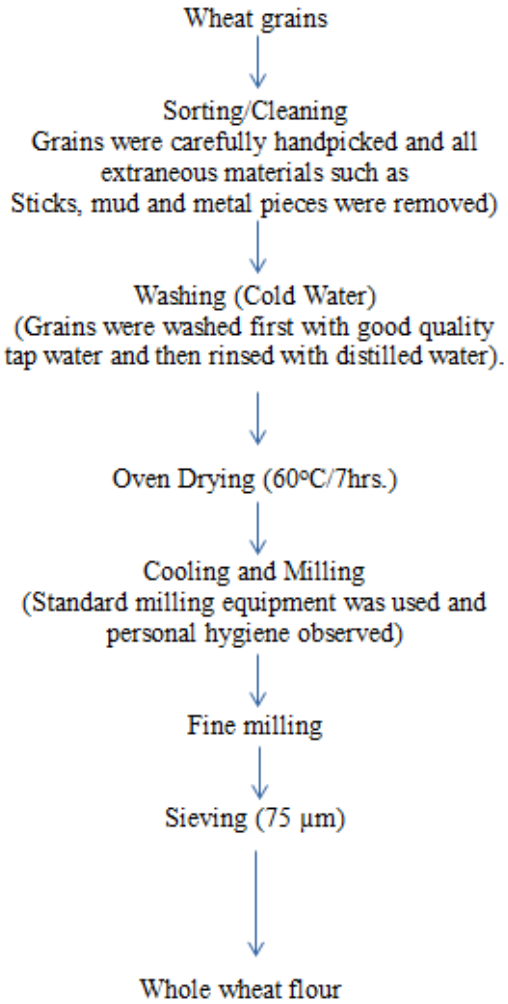

Figure 1:- Flow Chart for the Production of Whole Wheat Flour

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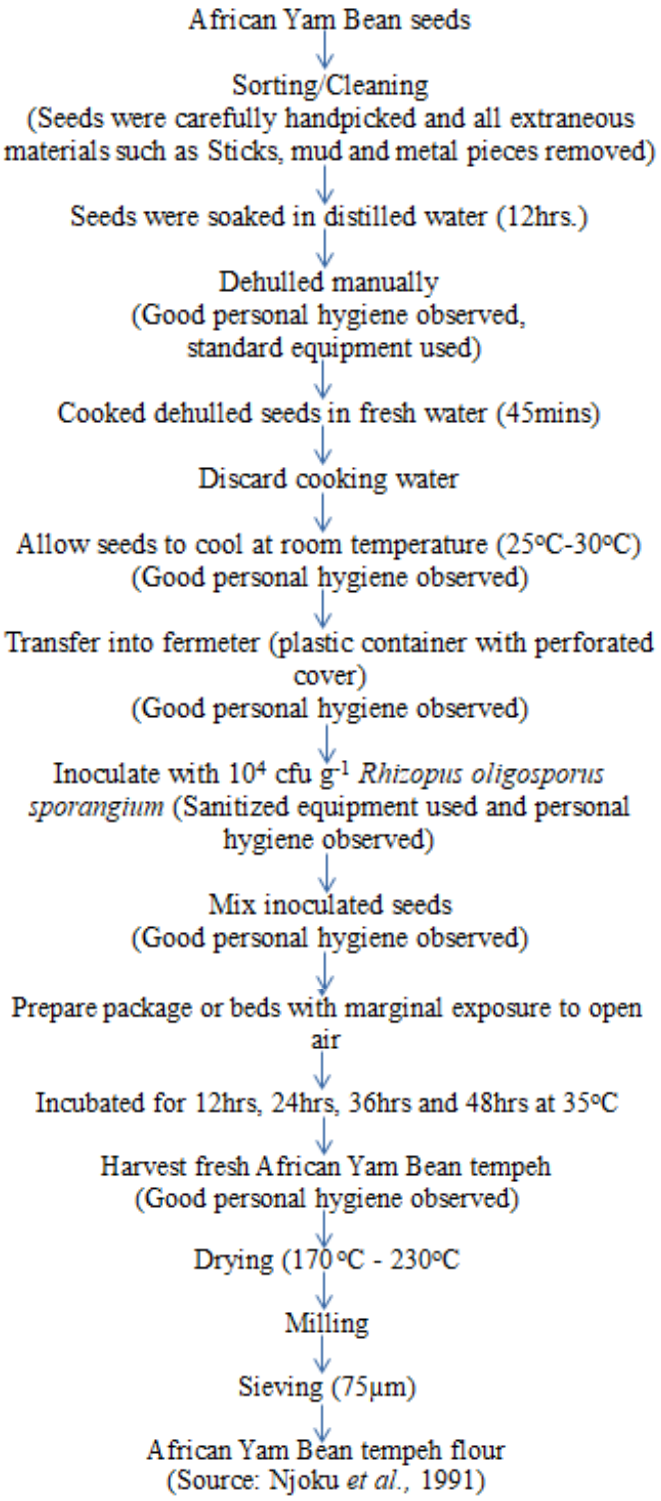

Figure 2:- Flow chart for the production of African Yam Bean Tempeh Flour

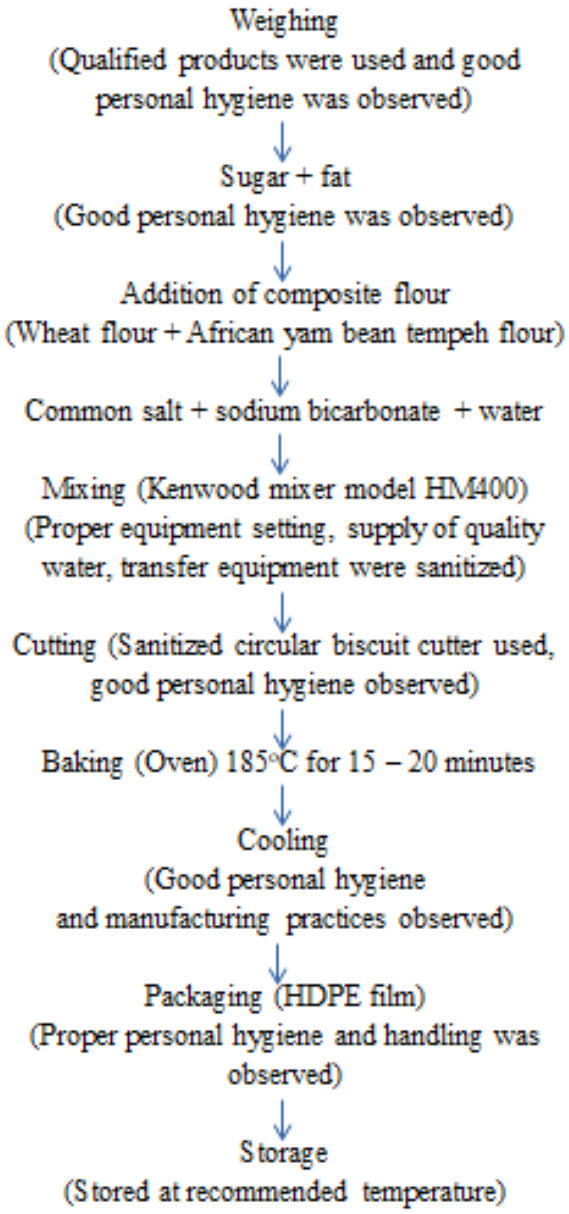

Figure 3:- Flow chart for biscuit production

➢ *Storage*

Biscuit samples were packaged and stored in polythene bags for five weeks at 28°C, and 80% relative humidity, prior to subsequent analyses and evaluation

➢ *Microbial Analyses*

The total viable count of the biscuit samples was determined using the pour plate technique as described by Giwa et al., (2012). Briefly, 1g of each sample was homogenized in 9ml of sterile peptone water in a sterile 500ml gas jar cylinder. The solution was agitated vigorously for a few minutes to ensure even mixing and then allowed to settle. A ten-fold dilution series was performed to obtain a colony count of $1x10^{-5}$(cfu/ml), one ml of the diluted sample was poured into an empty sterile 9ml Petri dish. Potato dextrose agar (PDA) was poured into the prepared plates for fungi counts, nutrient agar (NA) was poured into the

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prepared plates for bacterial counts, and MacConkey agar (MA) was poured into the prepared plates for enteric bacteria counts. All plates were prepared in duplicate. Colonies were counted and recorded after incubation period. The different colonies on each plate were isolated, purified and stored on nutrient agar slants (for bacteria) and PDA slants (for fungi) for further characterization and identifications.

➢ *Sensory Evaluation*

Sensory evaluation was carried out, in a sensory evaluation room. The biscuit samples were evaluated based on visual appearance, texture, taste, aroma, crust, shape and general acceptability. The evaluation was carried out by a semi trained 10-member panel, using a 9-point Hedonic scale with 1 representing the least score (where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely) was used (Akinjayeju, 2009).

➢ *Statistical Analyses*

The results obtained were subjected to analyses of variants (ANOVA), and Duncan multiple range test (Duncan, 1995) was used to separate means where significant difference existed.

Table 1:- Recipe for Biscuit Production

Sample	Wheat flour (%)	AYB Tempeh Flour	Salt (NaCl) (g)	Sugar (g)	Fat (g)	Sodium – bicarbonate baking powder (g)
A	100	0	1.0	30.0	20.0	2.0
B	90	10	1.0	30.0	20.0	2.0
C	80	20	1.0	30.0	2 0.0	2.0
D	70	30	1.0	30.0	20.0	2.0
E	60	40	1.0	30.0	20.0	2.0
F	50	50	10	30.0	20.0	2.0

Tables 2-8 shows microbial analyses of biscuits made from Wheat Flour and African Yam Bean Tempeh Flour.

Table 2:- Changes in Heterotrophic Count (cfu/g) of formulated biscuits
Storage period in weeks

Samples	Week 0	Week 1	Week 2	Week 3	Week 4
A	-	-	-	-	-
B	-	-	-	1.2 x 10	2.8×10^2
C	-	-	-	1.4 x 10	3.4×10^3
D	-	-	-	2.5×10^2	4.0×10^3
E	-	-	1.2×10^1	4.8×10^2	6.5×10^3
F	-	-	-	-	-

Table 3: Changes in Total Coliform Count (cfu/g) of formulated biscuits
Storage period in weeks

Samples	Week 0	Week 1	Week 2	Week 3	Week 4
A	-	-	-	-	-
B	-	-	-	-	-
C	-	-	-	1.2 x 10	1.3×10^2
D	-	-	-	-	-
E	-	-	-	1.1×10^2	1.5×10^2
F	-	-	-	-	1.0 x10

Table 4: Changes in Lactobacilli Count (cfu/ml) of formulated biscuits
Storage period in weeks

Samples	Week 0	Week 1	Week 2	Week 3	Week 4
A	-	-	-	-	1.0×10^2
B	-	-	-	1.3 x 10	2.1×10^2
C	-	-	-	2.0×10^2	2.7×10^2
D	-	-	-	2.6×10^2	1.6×10^3
E	-	-	1.0×10^1	3.7×10^2	6.8×10^3
F	-	-	-	-	-

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Table 5: Changes in Staphylococcal Count (cfu/ml) of formulated biscuits
Storage period in weeks

Samples	Week 0	Week 1	Week 2	Week 3	Week 4
A	-	-	-	-	-
B	-	-	-	2.0 x 10	1.8 x 10^2
C	-	-	-	3.5 x 10	5.4 x 10
D	-	-	-	3.5 x 10	5.5 x 10^2
E	-	-	-	4.5 x 10^2	5.7 x 10^2
F	-	-	-	-	-

Table 6: Changes in Proteolytic Organisms count (cfu/ml) of formulated biscuits
Storage periods in weeks

Samples	Week 0	Week 1	Week 2	Week 3	Week 4
A	-	-	-	1.2 x 10	-
B	-	-	-	-	1.0 x 10^2
C	-	-	-	-	1.7 x 10^2
D	-	-	-	3.0 x 10^2	3.5 x 10^2
E	-	-	2.0 x 10	3.1 x 10^2	4.3 x 10^2
F	-	-	-	-	-

Table 7: Changes in Mould Count (cfu/ml) of formulated biscuits
Storage period in weeks

Samples	Week 0	Week 1	Week 2	Week 3	Week 4
A	-	-	-	-	-
B	-	-	-	1.3 x 10	2.0 x 10^2
C	-	-	-	1.5 x 10	2.5 x 10^2
D	-	-	-	2.0 x 10	3.1 x 10^3
E	-	-	-	4.2 x 10^3	4.5 x 10
F	-	-	-	-	1.0 x 10

Table 8: Changes in Yeast Count (cfu/g) of formulated biscuits
Storage period in weeks

Samples	Week 0	Week 1	Week 2	Week 3	Week 4
A	-	-	-	-	1.1 X 10^3
B	-	-	-	2.5 x 10^2	1.4 x 10^3
C	-	-	-	3.1 x 10^2	1.6 x 10^3
D	-	-	-	4.3 x 10^2	1.8 x 10^3
E	-	-	-	5.4 x 10^2	2.5 x 10^3
F	-	-	-	-	5.1 x 10

Key:
A = 90% wheat flour / 10% African yam bean tempeh flour
B = 80% wheat flour / 20% African yam bean tempeh flour
C = 70% wheat flour / 30% African yam bean tempeh flour
D = 60% wheat flour / 40% African yam bean tempeh flour
E = 50% wheat flour / 50% African yam bean tempeh flour
F = 100% wheat flour / 0% African yam bean tempeh flour

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Table 9: Sensory evaluation scores of formulated biscuits stored at ambient condition for 5 weeks. (Mean±Stdev)

Attributes	Biscuit Samples	WK 0	WK 1	WK 2	WK 3	WK 4
Colour	A	8.00±0.10^{b}	7.04±0.02^{c}	7.05±0.02^{c}	7.99±0.09^{b}	8.05±0.02^{b}
	B	7.80±0.10^{c}	8.12±0.14^{b}	7.66±0.05^{b}	7.70±0.06^{c}	7.66±0.03^{c}
	C	7.00±0.10^{d}	7.00±0.10^{c}	7.00±0.00^{d}	6.91±0.01^{d}	6.80±0.10^{d}
	D	4.50±0.20^{e}	4.40±0.20^{d}	4.37±0.00^{e}	4.37±0.02^{e}	4.25±0.05^{e}
	E	3.00±0.00^{f}	2.98±0.01^{e}	2.97±0.00^{f}	2.90±0.01^{f}	2.50±0.05^{f}
	F	8.58±0.01^{a}	8.58±0.01^{a}	8.58±0.00^{a}	8.28±0.04^{a}	8.25±0.05^{a}
Aroma	A	7.30±0.05^{b}	7.30±0.05^{b}	7.30±0.05^{b}	7.20±0.05^{b}	7.15±0.05^{b}
	B	7.20±0.05^{b}	7.20±0.05^{b}	7.15±0.05^{b}	7.00±0.05^{c}	6.99±0.01^{c}
	C	7.00±0.10^{c}	7.00±0.10^{c}	7.00±0.10^{c}	6.98±0.10^{c}	6.90±0.05^{c}
	D	6.50±0.10^{d}	6.30±0.10^{d}	6.00±0.10^{d}	6.00±0.10^{d}	5.50±0.10^{d}
	E	4.50±0.10^{e}	4.20±0.10^{e}	4.00±0.10^{e}	3.85±0.10^{f}	3.50±0.10^{e}
	F	7.60±0.10^{a}	7.60±0.10^{a}	7.60±0.02^{a}	7.50±0.10^{a}	7.40±0.10^{a}
Texture	A	7.60±0.10^{a}	7.60±0.10^{a}	7.53±0.02^{b}	7.53±0.02^{a}	7.43±0.00^{b}
	B	7.00±0.10^{b}	7.00±0.10^{b}	7.00±0.10^{c}	6.90±0.20^{b}	6.90±0.10^{c}
	C	6.50±0.10^{c}	6.50±0.10^{c}	6.49±0.00^{d}	6.45±0.05^{c}	6.45±0.05^{d}
	D	5.50±0.10^{d}	5.30±0.10^{d}	5.20±0.10^{e}	4.80±0.10^{d}	4.10±0.11^{e}
	E	5.00±0.10^{e}	4.50±0.10^{e}	4.25±0.05^{f}	4.60±0.10^{e}	4.00±0.10^{e}
	F	7.70±0.10^{a}	7.70±0.10^{a}	7.70±0.10^{a}	7.60±0.10^{a}	7.60±0.10^{a}
Taste	A	7.00±0.10^{b}	7.00±0.10^{b}	7.00±0.10^{b}	6.90±0.10^{b}	6.88±0.02^{b}
	B	6.80±0.10^{c}	6.80±0.10^{d}	6.79±0.00^{d}	6.79±0.00^{b}	6.70±0.10^{d}
	C	6.50±0.10^{d}	6.50±0.10^{d}	6.50±0.10^{d}	6.40±0.10^{d}	6.30±0.10^{d}
	D	6.00±0.00^{e}	5.85±0.05^{e}	5.80±0.10^{e}	4.80±0.10^{d}	4.50±0.10^{e}
	E	5.00±0.10^{f}	4.90±0.10^{f}	4.50±0.10^{f}	3.50±0.10^{e}	2.35±0.00^{f}
	F	8.00±0.10^{a}	8.00±0.10^{a}	8.00±0.10^{a}	7.98±0.02^{a}	7.95±0.05^{a}
Shape	A	7.50±0.10^{b}	7.50±0.10^{b}	7.50±0.10^{b}	7.30±0.10^{b}	7.29±0.00^{b}
	B	6.50±0.10^{c}	6.50±0.10^{c}	6.49±0.02^{c}	6.49±0.02^{c}	6.48±0.03^{c}
	C	6.40±0.10^{c}	6.40±0.10^{c}	6.35±0.05^{d}	6.35±0.05^{d}	6.35±0.05^{c}
	D	5.60±0.10^{d}	5.45±0.05^{d}	5.40±0.10^{e}	4.80±0.10^{e}	4.50±0.10^{d}
	E	4.50±0.10^{f}	4.00±0.10^{f}	4.75±0.10^{f}	4.50±0.10^{f}	4.00±0.10^{f}
	F	8.00±0.10^{a}	8.00±0.10^{a}	8.00±0.10^{a}	7.95±0.05^{a}	7.90±0.10^{a}
Overall Acceptablility	A	8.00±0.10^{b}	8.00±0.10^{b}	8.00±0.10^{b}	7.95±0.0^{b} 5	7.90±0.10^{b}
	B	7.50±0.10^{c}	7.50±0.10^{c}	7.48±0.10^{c}	7.48±0.02^{c}	7.40±0.10^{c}
	C	6.50±0.10^{d}	6.50±0.10^{d}	6.50±0.10^{d}	6.45±0.05^{d}	6.45±0.05^{d}
	D	6.00±0.10^{e}	5.90±0.10^{e}	5.60±0.10^{e}	4.60±0.10^{e}	4.20±0.02^{e}
	E	5.10±0.11^{f}	5.00±0.10^{f}	4.50±0.10^{f}	3.60±0.11^{f}	2.70±0.02^{f}
	F	8.20±0.02^{a}	8.20±0.02^{a}	8.20±0.02^{a}	8.18±0.02^{a}	8.18±0.02^{a}

Scores are based on a 9 point hedonic scale where:
9 = like extremely
5 = neither like nor dislike
1 = dislike extremely.

III. RESULTS AND DISCUSSION

Table 9, shows the sensory evaluation of the biscuit samples produced from blend of WF and AYBTF. Biscuits produced from 100% WF had the utmost scores in crispiness (7.7), aroma (7.6), taste (8.0), shape (8.0), colour (7.6),it also showed a marked variation ($p < 0.05$) from biscuit samples made of fortified flour mixes, and it was generally more acceptable (8.2) by the panelist. Amongst the blended samples, a decrease of acceptability from sample A – E was observed, an indication that AYBTF impacted unfavorably on the savor of the biscuits particularly because of its beany flavor. Colour was another attribute of the biscuit impacted by AYBTF, as it made them much darker at higher AYBTF level. The dark colour may be as a result of the reaction between the reducing sugar and amino acids (Millard reaction). Though samples A – B were lighter in colour and appreciably differed ($p < 0.05$) from C – E. In congruence with this work, Sakyi – Dawson, et al, (2006) likewise noted darkening in colour of biscuits of cowpea-cassava flour-wheat flour composites. The texture of biscuit decreases with increase in the level of AYBTF in the blend, which

could be due to the increase in the crude fibre of African yam bean seeds in the blend. The result obtained is in line with the findings of Ferial et al., (2011). In terms of shape, Biscuits processed with only wheat flour (sample F) had the highest score (8.0) followed by samples A and B. The results of the microbial analyses of biscuit samples produced from blending WF and AYBTF is shown on tables 2 – 8. The microbiological analyses of biscuit revealed no visible growth observed on all the biscuit samples during the first three weeks of storage, as the biscuits were freshly produced, packaged under a good hygienic condition and the worth of the packaging material used was not compromised. Total viable count of bacteria and fungi showed growth on about all samples between the 3rd and 4th weeks of storage except for very few samples such as A and F in week 3 where visible growth was not observed, but on week 4, visible growth was noticed in all the samples. It was observed that, as the AYBTF increased in the composite, the microbial growth count likewise increased slightly. These results logically follow the position put forth by Adams and Moss (1999) where they reported that spoilage organisms grows faster in medium that is highly nutritious. Moreover the microbial load was also seen to have increased during the storage period, which indicates that the storage condition was not favourable for the biscuits, in congruence with the result of Butt et al. (2004), Sevaratnam et al., (2012) and Nagi et al. (2012).

Food production cannot be 100% free from microorganisms. With mean total count 1.0*10 – 6.8*103cfu/g, all biscuit samples had the allowable microbial limit of 104 to less than 106cfu/g of ready to eat food product (ICMSF, 2002). This shows that the biscuits are acceptable and it reflects a high hygiene standards adopted in the food preparation.

Staphylococcus was observed in all the biscuit samples, with increase in the mixes of the AYBTF, though less than the acceptable standard limit of 5.0*105 – 1.0*106cfu/ml, Eze et al., (2011). This is in congruence with the findings of Noah (2017) who observed Staphylococcal counts varying from 1.0*102 to 3.0*102cfu/g in biscuit made with coconut-almond fortified wheat flour. The average counts of coliform was 1.0*10 to 1.5*102cfu/g which is within the microbial limit standard for coliform in ready-to-eat-foods which is 102 – 103. This is in congruence with the observation of Saddazai et al., (2009), that coliform count in bakery product was within tolerable limits in biscuit. Therefore, maintenance of good manufacturing practices during production of bakery products so as to minimize microbial contamination should continue.

IV. CONCLUSION

This study has shown the microbiological evaluation of all biscuit samples are with in microbial limit standard of snacks. The sensory attributes of the biscuits clearly showed that biscuits made from the control (100% wheat flour) were generally acceptable by the panelist seconded by, biscuits produced from 90% wheat and 10% African yam bean tempeh flour (sample A) than the other biscuit samples. The research clearly showed the possibility of utilizing wheat flour fortified with African yam bean tempeh flour in producing confectionaries. The fermentation of AYB seeds to tempeh first, which has an attractive aroma and texture before milling into flour increases its awareness and help to over-power its beany savor which is one of the constraints that leads to its under-utilization in Nigeria.

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