ABSTRACT
A Key Step towards the Release of Genetically Modified (GM) Biofortified Sorghum is its Nutritional Evaluation and Risk Assessment Study. In this work, two genetically modified sorghum (ABS 188 and ABS 203) and local sorghum were subjected to proximate analysis, Amino acid determination, and mineral analysis. Results of proximate analysis revealed the presence of the following expressed as percentage (%) ABS 188 contains Crude fibre (1.89), Ash (1.87), Crude protein (9.00), Oil (1.36), Lignin (8.38) dry Matter (91.17) and Nitrogen free extract (77.05); ABS 203 contains crude fibre (1.60), Ash (0.95), Crude protein (4.94), Oil (2.56), Lignin (8.37) Dry Matter (91.63) and Nitrogen free extract (81.58); Local sorghum contains crude fibre (2.08), Ash (0.85), Crude protein (4.56), Oil (1.12), Lignin (8.28) Dry Matter (91.72) and Nitrogen free extract (83.11); respectively. ABS 203 has the highest amount of β Carotene (0.83ug/100mg), followed by 0.57ug/100mg for ABS 188 and 0.42ug/100mg for local sorghum. Results also revealed that the food samples contain almost all the essential amino acids in varying concentration, with glutamic acid occurring highest in the GM sorghum ABS 188 and ABS 203 having the highest for Leucine (14.01g/100g). Zinc was present in trace amount in all the samples (0.0003 ~ 0.033 mg/kg). In conclusion, ABS 188 and ABS 203 are considered to be as nutritious as local sorghum, with the advantage that the GM sorghum are biofortified with Vitamin A, Zinc and Iron.

Keywords: Genetically Modified Sorghum, Biofortification, Nutrition and Transgene

I. INTRODUCTION
Micronutrient deficiency or hidden hunger, which is characterized by chronic deficiency of essential vitamins and minerals such as vitamin A, iron, zinc and iodine, affects millions of people, especially the rural poor and other vulnerable populations. In Sub-Saharan Africa, where the highest prevalence of hidden hunger is recorded, vitamin A deficiency alone affects 48% of children under five years while iron deficiency is responsible for many cases of anaemia affects 63% of children under 5 years, contributing to 20% of all maternal deaths. Furthermore, about one-third of the world’s population suffer from zinc deficiency while 26% of Africa’s population is at risk of becoming zinc deficient. The most significant and common clinically micronutrient deficiencies in children and women of childbearing age include deficiencies of iron, iodine, zinc, and vitamin A and are estimated to affect as many as two billion people (Luchuuet al, 2013). Sorghum [Sorghum bicolor (L.) Moench], a tropical plant belonging to the family of Poaceae, is one of the most important crops in Africa, Asia and Latin America (Anglani, 1998). In terms of total cereal consumption, sorghum represents about 30% (USAID, 2010) Nigeria is the largest cereal producer in West Africa accounting for about 71% of the regional sorghum production in 2006 (RECA Niger, 2010). Therefore, sorghum plays a crucial role in contributing to house-hold food security in many of the world’s poorest and most food-insecure regions, that cannot afford imported rice and wheat-based food (ICRISAT/FAO, 1997). Sorghum grain has a nutritional profile similar to corn and other cereals as it shares the typical nutritional deficiencies of cereal grains, a low content of several essential amino acids, a low vitamin A and E content and a low bioavailability of iron and zinc (Shewry and Halford, 2003). Therefore, a diet, based mostly on sorghum, is not adequate to meet the nutritional growth or maintenance requirements for children and adults and needs to be supplemented with essential amino acids and micronutrients. Advances in genetics and molecular biology have enabled the development and commercial release of genetically modified organisms (GMOs) such as sorghum, with traits that transcend the species barriers. The development of biofortified sorghum offers the potential for increased agricultural productivity or improved nutritional values that can contribute directly to enhancing human health and development.

Biofortification is a process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or genetic modification. Howarth and Amy (2017) reported
that biofortified crops can improve human nutrition and address micronutrient deficiencies by increasing the daily adequacy of micronutrient intakes among individuals throughout the lifecycle. Biofortified crops are also a feasible means of reaching rural populations who may have limited access to diverse diets or other micronutrient interventions (Howarth et al., 2011).

Genetically modified plant has been one of the most rapidly adopted technologies in the history of agriculture. (Harlander, 2002). Since the introduction of the first genetically modified plant in the 1983, genetic engineering techniques and their applications have developed rapidly. The development of the nutritionally enhanced sorghum lines will rely on transgenes and technologies that have shown high efficacy in transgenic maize and that resulted in a significantly improved nutritional quality of maize grain. As a proof of concept, a first-generation transgenic sorghum line (ABS #1) that possesses grain with a 50% increase in lysine has already been developed. The goal of the ABS project is to develop transgenic sorghum varieties that will overcome most of the described nutritional deficiencies by substantially improving grain digestibility, by delivering vitamins, the essential amino acids lysine, threonine and tryptophan, and by improving the bioavailability of iron and zinc. (Zhao et al., 2003)

The main objective of this study was to assess the nutritional value of genetically modified sorghum through proximate analysis, mineral analysis, Amino acid profiling and carotene determination.

II. MATERIALS AND METHODS

Source of Sorghum Bicolar

The Genetically Modified sorghum were obtained from The National Biosafety Management Agency Abuja, through institute for Agricultural Research Zaria (IAR). The local sorghum was obtained from the Central market Kaduna and was identified in National research institute for chemical technology (NARICT) Zaria with voucher number 0/2018.

Proximate Analysis

The proximate analyses of the samples for moisture, total ash, crude fibre, crude fat, carbohydrate and crude protein were carried out using the method described by AOAC (1980). The Nitrogen content was determined by Micro Kjeldahl method described by James et al., (1999), involving digestion, distillation and finally titration of sample.

Carotene Determination

Carotenoid determination was carried out according to Rodriguez-Amaya (2001). Two grams (2g) of ground fresh samples were mixed with 50ml of acetone until loss of pigmentation. The mixture obtained was filtered and total carotenoids were extracted with 100ml of petroleum ether. Absorbance of extracted fraction was then read at 450nm. Total carotenoids were subsequently estimated using a calibration curve of B-carotene (1mg/ml) as standard.

Mineral Analysis

Exactly 10g of samples was dried in a muffle furnace at 550°C for 5hours until a white residue of constant weight was obtained. The minerals were extracted from ash by adding 20ml of 2.5% HCl, heated in a steam bath to reduce the volume to 7ml, and this was transferred quantitatively to a 50ml volumetric flask. It was diluted to a volume of 50ml with deionized water, stored in clean polyethylene bottles and mineral contents was determined using an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, USA). Sodium and Potassium were determined using flame photometry (Mustapha and Magdi, 2003). Iron, calcium, magnesium and zinc were analyzed by the method of Sakamoto et al (2008).

Amino Acid Profiling

The Amino Acid profile in each sample was determined using methods described by AOAC (2006), and Benitez (1989). The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH (phenylthioohydantoin) Amino Acid Analyzer.

III. RESULTS

Proximate Analysis of the Three Varieties of Sorghum and Standard Diet

The proximate composition of African biofortified sorghum (ABS188 and ABS203) and local sorghum bicolar are shown in Table 4.1. It was observed that in the experimental groups, ABS 188 has the highest amount of crude protein (9.0%), followed by ABS 203 and local sorghum with a value of 4.94% and 4.56% respectively, while the standard diet has the highest value of (10.75%). The crude fiber content ranged from 1.68 – 6.10% with Standard diet having the highest value. The lignin of the samples were almost the same, ranging from 8.28 – 8.83%, except for the standard diet which has a value of 11.41%. The oil content of ABS 203 was 2.56% which is significantly higher than that of ABS188 with a value 1.36% and local sorghum which has a value of 4.92%. The free Nitrogen and Dry matter where relatively higher in the experimental groups, ABS 188 with a value 11.7% and local sorghum which has a value of 10.75%, although the standard diet has the highest value of 4.92%. The free Nitrogen and Dry matter where relatively higher in the three sorghum varieties than in the standard diet with a value ranging from 77.05 – 83.11% for the free nitrogen and a value of 91.17 – 91.72 for the Dry matter.
Table 4.1: Proximate Analysis of the Three Varieties of Sorghum and Standard diet

| Features            | ABS 188 | ABS 203 | Local Sorghum | Standard diet |
|---------------------|---------|---------|---------------|---------------|
| Lignin %            | 8.83    | 8.37    | 8.28          | 11.41         |
| Crude Protein %     | 9.00    | 4.94    | 4.56          | 10.75         |
| Crude fibre %       | 1.89    | 1.60    | 2.08          | 6.10          |
| Oil %               | 1.36    | 2.56    | 1.12          | 4.92          |
| Ash %               | 1.87    | 0.95    | 0.85          | 7.21          |
| Nitrogen free Extract % | 77.05 | 81.58  | 83.11    | 59.61         |
| Dry Matter %        | 91.17   | 91.63   | 91.72         | 88.59         |

Carotenoid Determination

β-carotene concentration of local sorghum and African biofortified sorghum event 188 and event 203 is shown in figure 1. The β – Carotene content of all the three sorghum samples ranges between 0.42ug/100mg to 0.83ug/100mg with ABS 203 having twice as much as that of local sorghum.

![Figure 1: β-Carotene concentration of the sorghum varieties.](image)

Mineral composition of the three sorghum varieties

The mineral composition of ABS 188, ABS 203 and local sorghum is presented in table 4.2, which reveals that the concentration of sodium is high ranging from 1.0293 – 1.0821 mg/kg. There was a trace amount of zinc in the samples with values ranging from (0.0006– 0.0033 mg/kg). Local sorghum has highest values for Potassium and iron with a value of 2.7174mg/kg and 0.5350mg/kg respectively. While ABS203 has the highest values for Manganese and Magnesium.
Table 3. Mineral Analysis of three sorghum varieties in mg per Kg.

| Minerals | ABS 188 | ABS 203 | Local Sorghum |
|----------|---------|---------|---------------|
| Na       | 1.0821  | 1.0717  | 1.0293        |
| K        | 0.7759  | 0.4493  | 2.7174        |
| Fe       | 0.2985  | 0.3457  | 0.5350        |
| Ca       | 0.0628  | 0.0520  | 0.0451        |
| Mg       | 0.0127  | 0.6706  | 0.0127        |
| Zn       | 0.0033  | 0.0006  | 0.0033        |
| Mn       | 0.0207  | 0.0368  | 0.0247        |

Amino acid Composition of the Three Sorghum Varieties

Results of amino acid composition showed that all the sample contain almost all the essential amino acids in varying concentration (Table 4.3). Glutamic acid content ranging from (18.92 – 20.14 g/100g), proline ranging from (8.32 – 9.44g/100g), Tyrosine ranging from (3.87 – 4.3g/100g), and Aspartic acid ranging from (6.95 – 7.32g/100g) occurred highest in ABS 188. ABS 203 has the highest concentration for Leucine ranging from (11.38 – 14.01g/100g), phenylalanine ranging from (4.79 – 5.53g/100g), Arginine ranging from (3.78 – 4.3g/100g), Valine ranging from (4.97 – 5.5g/100g), Methionine ranging from (1.6 – 2.08g/100g), Cystine ranging from (1.82 – 2.18g/100g) and Alanine ranging from (8.87 – 9.63g/100g), while Local sorghum has the highest value for threonine, and isoleucine.

Table 4.3: Amino acid Composition of the Three Sorghum Varieties. (g/100g protein)

| Amino Acid       | ABS 188 g/100g | ABS 203 g/100g | LOCAL g/100g | 2002 Estimate RDA |
|------------------|----------------|----------------|---------------|-------------------|
| Leusine          | 12.02          | 14.01          | 11.38         | 0.059             |
| Lysine           | 2.33           | 2.39           | 2.01          | 0.045             |
| Isoleucine       | 3.86           | 3.93           | 3.99          | 0.03              |
| Phenylalanine    | 4.79           | 5.53           | 4.97          | 0.038             |
| Arginine         | 3.78           | 4.3            | 3.96          |                   |
| Tryptophan       | 1              | 1.21           | 1.1           | 0.006             |
| Valine           | 5.2            | 5.5            | 4.97          | 0.039             |
| Threonine        | 3.05           | 3.22           | 3.5           | 0.023             |
| Histidine        | 2.17           | 2.11           | 2.11          | 0.015             |
| Non-essential    | Amino acid     |                |               |                   |
| Proline          | 9.44           | 8.93           | 8.32          |                   |
| Tyrosine         | 4.13           | 3.87           | 3.96          |                   |
| Cystine          | 1.82           | 2.18           | 1.94          | 0.006             |
IV. DISCUSSION

Genetic modification technology ensures improvement in productivity and quality of crops (Hashimoto et al., 1999). Guidelines for the safety or risk assessment process to demonstrate that GM crops are as safe as the conventional non-GM crops, requires proximate analysis of key nutrients in GM crops and Feeding studies, to compare the nutritional performance of GM crops to non-GM crops (WHO, 1995).

The proximate composition of the genetically modified sorghum (ABS 188 and ABS 203) and the local sorghum has appreciable amount of the component with the ABS 188 having the highest percentage of crude protein and Ash. This result is in consonance with the findings of George et al. (2004) and Adebiyi (2005) who found out that the crude protein content of genetically modified maize and sorghum was higher than that of local maize. The crude fibre content of local sorghum (2.08%) was slightly higher than that of ABS 203 (1.6%) and ABS 188 (1.89%), which agrees with the result of Udachan et al. (2012), who reported a value of 1.9% and 2.7% for the dadar and CSH-9 sorghum respectively. The oil was in the range of 0.40 – 2.7%, the maximum oil percentage was found in ABS 203 followed by ABS 188, this is in agreement with the findings of Udachan et al. (2012), who reported a value range of 2.30 – 2.8%. ABS 203 has twice as much β-carotene as the local sorghum and also that of ABS 188 is slightly higher than the local sorghum this is in agreement with the report given by Africa Harvest (2018). These may be due to the fact that the two genetically modified sorghums were genetically modified by introducing two genes that enhance the production of β-carotene a precursor for vitamin A which is an essential vitamin that support cell growth, immune function, fetal development and vision. The non-difference in the nitrogen, lignin and dry matter of the genetically modified sorghum when compared with that of local sorghum, is in consonance with the findings of George et al. (2004).

Amino acid analyses have been carried out on hybrids of grain sorghums (Herman et al., 2004). The presence of some essential amino acids in varying proportions may be an indication of the better nutritional value of the sorghum (George et al., 2004). Sorghum have been proven to have reduced amounts of lysine, threonine and sulphur containing amino acids (Nandini and Salimath, 2001), which agrees with the result of this study. In consistency with the findings of Torres et al. (2006), GM sorghum and local sorghum contain relatively high amount of glutamic acid and Leucine with ABS 203 having the highest value for luecine and ABS 188 for Glutamic acid. According to the ABS project technology development, the transgenic sorghum has an improved protein profile; tryptophan (10-20%), lysine (30-120%), Threonine (30-40%) Africa Harvest (2018).

The results of the elemental mineral determination indicated the presence of sodium, potassium, calcium, magnesium, iron and zinc in all the samples; their presence in the samples cannot be underrated because of their role in health and nutrition. The transgenic sorghum (ABS188 and ABS 203) are biofortified with β-carotene, zinc and iron, by inserting a gene which code for the nutrients. All the three samples ABS188, ABS 203 and local sorghum have high level of iron and low concentration of zinc. The bioavailability studies carried out on the transgenic sorghum have shown the presence of zinc (30-40%) and iron (20-30%) Africa Harvest (2018). The result shows various concentrations of the minerals for the three samples which is in contrast with the findings of Jimo and Abdullahi (2017). The difference may be attributed to storage of the raw materials.

There is a growing concern that introducing foreign genes into food plants may have an unexpected and negative impact on human health, hence animal experiment is employed in this study. Animal feeding experiment gives valuable and reliable information regarding the safety of a Genetically modified plants for both livestock and human consumption (Alexander et al., 2007). Currently, genetically modified varieties of yellow maize, soybean and sorghum are produced for animal feed (Nowicki et al., 2010).

V. CONCLUSION AND RECOMMENDATION

The proximate analysis revealed that genetically modified sorghum (ABS 188 and ABS 203) and local sorghum contain appreciable amount of nutrients, and
their compositions are similar. The amino acid and mineral composition of the three sorghum samples are not significantly different. Molecular analysis showed that there is presence of transgenes (ZmPsy and CrT1genes) in the genetically modified sorghum (ABS 188 and ABS 203) but absent in the local sorghum.

In view of this, ABS 188 and ABS 203 are considered to be substantially equivalent to, and as nutritious as local sorghum.

Lack of dietary diversity and low intake of many macro and micro-nutrients have led to serious malnutrition; hence the need for biofortified and genetically modified food. Because the consumption of genetically modified food has been on the increase and gradually being accepted by diverse people, it is paramount for risk assessment to be performed on other GM crop species in order to ascertain their potential impact in near future.

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