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EVALUATION OF NUTRITIVE AND ANTI-NUTRIENT CONTENTS OF COMPOSITE MEAL FROM *Sorghum bicolor* (SORGHUM), *Glycine max (*SOYA BEANS) AND *Sesame* (BENNI SEEDS)

[1]FALMATA, A. S., [1]SIHIYONA, E. W., [2]MARYAM, B. K., [1]BINTU, B. P., [2]SALAMATU, T., *CHAMBA, G. AND MODU, S.

[1]Department of Biochemistry, University of Maiduguri, Borno State, Nigeria.
[2]Department of Biological Science, University of Maiduguri, Borno State, Nigeria.

*Corresponding Author: gonichamba97@gmail.com

ABSTRACT

Composite meal is a meal made up of different classes of food such as carbohydrate, protein, lipids, which can be used for the management of Protein and Energy Malnutrition (PEM). The study evaluates the nutritive and anti-nutritive contents of composite meals produced from Sorghum, Soya beans and Benni seeds. The materials (sorghum, soya beans and benni seeds) used were purchased from Maiduguri Monday Market, Borno State, Nigeria. They were subjected to standard processing methods. The study investigated the proximate composition and antinutritional factors of the samples. Two different ratios used, were 60% and 50% sorghum, 40% and 30% soya beans and 20% benni seeds. The results of the proximate analysis revealed that there were significant (p<0.05) increases in protein, fat and fibre contents of the composite meal particularly, the 50:30:20 ratios; sorghum, soya beans and benni seeds respectively. The results of the anti-nutrient analysis showed a significant (p< 0.05) reduction in tannin and phytic acid in the composite meal. Based on the results obtained, the composite meal from sorghum, soya beans and benni seeds had a high nutritional value and can be used to ameliorate protein energy malnutrition.

Keywords: Benni Seeds, Composite Meal, Soya Beans And Sorghum

INTRODUCTION

Protein-energy malnutrition (PEM) or protein-calorie malnutrition refers to a form of malnutrition where there is inadequate calories or protein intake. It is a wide spread nutritional disease in the developing countries (Franco *et al.*, 1999). The World Health Organization (WHO) defines malnutrition as "the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions". The term protein-energy malnutrition (PEM) applies to a group of related disorders that includes; marasmus, kwashiorkor and intermediate states of marasmus-kwashiorkor. The term marasmus is derived from the Greek word '*marasmus',* which means withering or wasting. Marasmus involves inadequate intake of protein and calories and is characterized by emaciation. The term kwashiorkor is taken from the Ga language of Ghana and means "the sickness of the weaning". Williams first used the term in 1993, and it refers to an inadequate protein intake with reasonable caloric (energy) intake. Edema is characteristic of kwashiorkor but is absent in marasmus.

Protein-energy malnutrition (PEM) is still a major public health issue in developing countries (Muller *et al.,* 2005). It is associated with as 50-60% of under-five mortality in poor countries (Faruque *et al.,* 2008) and a myriad of morbidities. There are various anthropometric variables for classifying protein energy malnutrition (Gernaat *et al.,* 2000). Acute malnutrition, for instance, is

measured by weight for height or bilateral edema, while chronic malnutrition is measured by height for age. The WHO recently defined Severe Acute Malnutrition by a very low weight for height, visible severe wasting, or the presence of nutritional edema (WHO, 2011). Wasting (marasmus) and various forms of kwashiorkor are, therefore, forms of severe Acute Malnutrition. One of the oldest classifications of protein energy malnutrition (Welcome Working Group) used weight for age and the presence or absence of edema to arrive at a spectrum, with marasmus and kwashiorkor at either end of the spectrum (Gernaat *et al.,* 2000). Besides macronutrient deficiency, deficiencies in iron, iodine, vitamin A, and zinc are the main manifestations of malnutrition in developing countries, and indirect factors such as high rate of unemployment, poverty, illiteracy, and overcrowding contribute to the development of protein energy malnutrition (Muller *et al.,* 2005).

Other research works have been carried out on the evaluation of nutritive value of various composite nutrients in Nigeria, examples are the blend of cereals and legumes (Solomon, 2005), pearl millet and cowpea (Modu *et al.*, 2010) etc. Other worker, made composite from combination of sorghum for its protein, vitamins, and mineral content (Decardoso *et al.*, 2015), soya beans (38-40%) for protein, was found to be much higher than legumes and cereals (Bressani, 1981), and sesame seeds which is rich in methionine (Ojiako *et al*., 2010) was considered.Therefore, the primary aim of this study was to formulate a composite meal from sorghum, soya beans and sesame seed, and chemically evaluate their respective nutritional values so as to improve the protein quality of traditionally prepared complementary food blends from cereal and legumes, in various proportions to meet the dietary needs of the target population with a view to enriched the levels of essential amino acids to ameliorate protein energy malnutrition.

MATERIAS AND METHODS

Plant Materials

The sorghum (*Sorghum bicolor*), sesame and soya beans (*Glycine max*) were used to formulate the composite feed used in this study. The food stuffs were purchased from Maiduguri Monday market, there was no information about the length of storage of the sample before purchase.

Sample Preparation

The sorghum grains were first subjected to cleaning, which involves the removal of stones and unwanted particles. After cleaning, 300g of the sorghum grains were weighed and soaked in water about three times their weight by volume for 72 hours for fermentation to take place. After that the grains were thoroughly washed with clean water, sundried and milled to powder. The soy beans were also subjected to cleaning, after that 300g of the soya beans were weighed and then roasted for 15 minutes at 30^{o}C, then cooled and milled to powder. The benni seeds were subjected to cleaning. After cleaning, 300g of the benni seeds were weighed and washed thoroughly using clean tap water, sundried and then roasted for 15 minutes. After roasting the benni seeds were cooled and milled using a grinding machine

Proximate Analysis

The proximate analysis carried out on the composite meal were to determine the moisture content, dry matter, crude protein, crude fibre, ether extract or fat, ash and carbohydrate using the method of AOAC (2000).

Determination of Dry Matter

The dry matter content was determined by weighing 10g of each of the sample into petri

dish and then placed in hot oven at 105°C. After 24 hours the samples were removed, cooled in desiccator and reweighed. The dry matter content was calculated using the formula below: -

$$\frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where:

W_2 = weight of petri dish with sample in grams before oven dried

W_3 = weight of petri dish with sample in grams after oven dried

W_1= weight in grams of empty petri dish

Determination of Crude Protein

Crude protein content was analyzed using kjeldhal method. 2g of samples was weighed into a digestion tube and 2 kjeldhal tablets and 20 ml of concentrated sulfuric acid (H_2SO_4) were added into the tube and digested at 420°C for 3 hours. After cooling, 90 ml of distilled water was added into the digested solution. About 50 ml of 40% caustic soda was added onto 50 ml of digested and diluted solution and then placed on heating section of the distillation chamber, 30 ml of 4% boric acid, plus bromocresol green and methyl red as an indicator was put into conical flask and placed underneath the distillation chamber for collection of ammonia, the solution change from orange to green colour. About 0.1 normal solution of hydrochloric acid was weighed into burette. The conical flask containing the solution was titrated until the colour changes from green to pink. The burette reading was taken. The crude protein was calculated using the formula:

$$\% \text{ crude protein} = \frac{(\mathrm{A} - \mathrm{B}) \times \mathrm{N} \times \mathrm{F} \times 6.25}{\text{Mg of samples}} \times 100$$

Where: -

A = amount of acid used for titrating the sample

B = amount of acid used for titrating blank sample

N = normality of acid used for titration

F = factor of 14.007

Determination of Crude Fibre

Crude fibre was determined by weighing 2g of samples and then placed into a round or flat bottom flask and 50 ml of trichloroacetic acid was added. The mixture was then boiled and refluxed for 40 minutes. Filter paper was used to filter the residue. The residue obtained was washed four times with hot water and only once with petroleum ether. Then the filter with the sample were folded together and dried at 30-60°C in an oven for 24 hours. After drying, the filter with the sample was reweighed and then ash at 650°C and then cooled and reweighed.

$$\%\mathrm{CF} = \frac{\text{Difference in weighing}}{\text{weight of sample}} \times 100$$

Determination of Ether Extract (Fat)

The ether extract was determined by using Soxhlet apparatus. 2g of the sample was weighed into a thimble and 200 ml of petroleum ether measured using measuring cylinder was added. The solution was transferred into a round or flat bottom flask and was heated at 45°C for one hour. The flask was then removed, and cooled into a desiccator for 15 minutes. Percentage fat of the sample was determined using the formula: -

$$\% \text{ Fat} = \frac{\text{Weight of fat}}{\text{Weight of sampe}} \times 100$$

Determination of Ash

The ash content was determined by measuring 2g of the sample into a crucible and then dried at 105°C for 24 hours, then cooled in the desiccator for 15 minutes and reweighed. It was then combusted at 600°C in muffle furnace for 2 hours, then cooled in a desiccator for 15 minutes and reweighed. The ash content of the sample was determined using the formula below;

$$\% \text{ Ash} = \frac{\text{loss in weight}}{\text{Initial weight}} \times 100$$

Determination of Moisture Content

A clean flat crucible was dried in an oven and cooled in a desiccator. The cooled dish was weighed (W_1) and 5g of the sample was weighed into the dish and weighed accurately (W_2). The dish and its content was then transferred into an air oven to dry at 105°C for 3 hours. The dish was transferred into a desiccator using a pair of tongs and then allowed to cool and reweighed (W_3).

$$\% \text{ Moisure} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Determination of Carbohydrate

The carbohydrate content of each sample was determined by difference using the formula: -

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \text{Crude fat} + \% \text{ Protein})$$

Determination of Anti-Nutritional Factors

Anti-nutritional factors were determined in the composite meal. The anti-nutrients determined were phytic acid and tannins.

Determination of Phytic Acid

Phytic acid (inositol hexaphosphate) content were determined according to the method described by AOAC (1990).

Phytate is extracted using dilute HCl and then extract mixed with Na_2 EDTA- NaOH solution and placed in an ion exchange column. The extracted phytate is diluted with 0.7ml NaCl solution and wet – digested with H_2SO_4 mixture to release phosphate, which is measured calorimetrically after reacting with ammonium molybdate solution. The amount of phytate in original sample is obtained as hexaphosphate equivalent.

Determination of Tannin

Tannin content of the samples were determined by the method described by Price and Butler (1977). 0.2g of each sample was weighed into Erlenmeyer flask and 10 ml of 4% HCl in methanol was added. The flask was closed with paraffin wax and shaken for 20 minutes on a wrist action shaker. 1 ml of the extract was pipetted and 1 ml of 1% vanillin was added followed by 0.5 ml of concentrated HCl. Phenol standard solution was prepared by pipetting 0.1, 0.3, 0.5, 0.7 and 1.0 ml of phenol reagent into 5 different test tubes, the volume of the test tubes was made up to 1 ml of 1% vanillin was added and then 0.5 ml concentrated. HCl into each of the test tubes and the volume was made up to 5.5 ml of 4% HCl in methanol. The blank sample was prepared by using 5 ml of 4% HCl in methanol. The absorbance of the standard solutions, sample extract and sample blank were read using spectrophotometer at 500nm exactly 20 minutes after incubation.

$$\frac{\text{Au}}{\text{Cu}} = \frac{\text{Atd}}{\text{Cstd}}$$

Where Au = Absorbance of unknown
Astd = Absorbance of standard
Cu = concentrations of unknown
Cstd = concentration of standard

Statistical Analysis

Data collected during the study were subjected to analysis of variance and statistics version 8.0, American product was used to compare the means.

RESULTS AND DISCUSSION

Proximate composition of the composite meal from Sorghum, Soya beans and Benni seed

Table 1 shows the result of proximate composition of the composite meal. It is evident in the result that the samples do not differ significantly (P>0.05) from each other for dry matter and ash. For the protein content, they are significantly (P<0.05) different from each other, with the least (3.41%) content, in unprocessed sorghum and highest (10.14%) in fortified sorghum with soya beans and benni seeds. The result of carbohydrate also shows significant differences (P<0.05) with the highest (86.82%) content in unprocessed sorghum and least (68.397%) content in fortified sorghum with soya bans and benni seeds. However, for the moisture content, there is no significant (P>0.05) difference among processed sorghum, unprocessed sorghum and fortified sorghum with soya beans, but differs significantly (*P*<0.05) from that of fortified sorghum with soya beans and benni seed which has the least (2.7%) content. There is also a significant difference (P<0.05) among the fibre content of the samples with the least content (4.9%) in unprocessed sorghum, in unprocessed and highest content (16.5%) in fortified sorghum with soya beans and benni seeds. However, for the fat content there is no significant difference between processed sorghum and unprocessed sorghum, but differs significantly (P<0.05) from fortified sorghum with soya beans and benni seeds and fortified sorghum with soybeans respectively.

Table 1: Proximate Pomposition of the Composite meal from Sorghum, Soya beans and Benni seeds (%)

Samples	Drymatter (%)	Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Carbohydrate (Kcal/100g)
US	96.23 ± 0.29^{a}	$3.20 \pm (\%)0.12^{ab}$	3.4133 ± 0.07^{d}	2.03 ± 0.15^{c}	4.90 ± 0.06^{d}	1.20 ± 0.12^{a}	86.82 ± 0.13^{a}
PS	95.80 ± 0.42^{a}	3.43 ± 0.88^{a}	4.400 ± 0.10^{c}	2.03 ± 0.15	6.03 ± 0.09^{c}	1.13 ± 0.12^{a}	84.41 ± 0.28^{b}
FSS	96.47 ± 0.18^{a}	3.13 ± 0.07^{b}	5.4533 ± 0.25^{b}	4.76 ± 0.09^{c}	14.0 ± 0.35^{b}	1.23 ± 0.09^{a}	75.56 ± 0.31^{c}
FSSB	95.87 ± 0.75^{a}	2.70 ± 0.058^{c}	10.143 ± 0.22^{a}	4.83 ± 0.15^{a}	16.5 ± 0.29^{a}	1.06 ± 0.07^{a}	68.40 ± 0.26^{a}

Values are expressed in Mean ±SEM, n=3. Values in the same row with different superscript are significantly different (p<0.05).

Key words: -

US: Unprocessed sorghum
PS: Processed sorghum
FSS: Fortified sorghum with soybeans
FSSB: Fortified sorghum with soybeans and benni seed
SEM: Standard Error of Mean

The raw samples had low moisture content. This shows that food blends from this source will have a low moisture content, and longer shelf-life. This is because food spoilage microorganism can thrive in where there is high moisture contents. Similar work reported by Modu *et al*. (2010) and Bintu *et al*. (2012).

An increase in the protein content of the processed samples observed was probably due to fermentation. Chava *et al*. (1988) reported an increase in some soluble amino acids of sorghum within the first 24 hours of fermentation by proteolytic bacteria

The relatively low-fat content of the composite blends makes them suitable raw materials in the formulation of a variety of food products for the elderly (WHO, 2018). The fat contents of a food can affect its shelf life stability. This is because fat can undergo oxidative deterioration, which leads to food

spoilage. Hence, the food blend with a high fat content is more liable to spoilage than one with o lower fat content. However. The fat contents of all the food blends fall within the RDA range of infants 0-1 Year.

High dietary fibre contents have been reported to impair protein and mineral digestion and absorption in human nutrition (Bernard, *et al.,* 2016). Hence, low fibre blend are suitable for the adequate intake of protein and mineral. The fibre content of the blends reported in this study were higher than the FAO/WHO limits of <5%, possibly due to inclusion of soya beans which was reported to have high fibre content (USDA, 2020). The carbohydrate contents of the food blends were higher than the lower limit for carbohydrates (41.3-73.79 g/100g) of the Codex Alimentarius standard (FAO/WHO, 2017).

Anti-nutritive content (phytic acid and tannins) of the composite meal From Sorghum, Soya beans and Benni seeds.

Table 2 present the anti-nutritive content (phytic acid and tannins) of the composite meal From Sorghum, Soya beans and Benni seeds**.** The results reveals that the phytic acid content of the unprocessed sorghum is significantly different ($P<0.05$) from the other samples which has the highest content (0.66%). However, processed sorghum do not differ from fortified sorghum with soya beans, but differs significantly from fortified sorghum with soya beans and benni seeds which has the least (0.54%) content. Similarly, for tannin all the means differs significantly ($P<0.05$) from each other, with the least (0.3167%) content in fortified sorghum with soya beans and benni seeds.

Most cereals grains contain appreciable amounts of phytate while cereals like sorghum and millet contain significant amounts of polyphenols and tannins. The occurrence, chemical nature and mechanism of anti – nutritional or toxic effects of such compounds are well documented. Hence their reduction or total elimination through suitable processing methods becomes important in cereal – based foods (Chavan and Kadam; 1989).

Natural fermentation was observed to significantly reduce or totally eliminate certain anti – nutrients in cereal and cereal legume blends. (Chompreeda and fields, 1984). The reduction of phytate phosphorus and flatus sugars or trypsin inhibitor activity has been attributed to microbial degradation of these compounds.

The reduced levels of phytate and tannins in the processed samples of cereals and legumes could be as a result of the leaching effect of the soaking and dehulling employed on samples before milling (Falmata *et al.,* 2014). The removal of seed coat (dehulling) to soak cowpea and groundnut, might be attributed to the reduction of the phytic acid content of the roasted cowpea and groundnut. Soaking of cereals and legumes usually forms an integral part of processing methods such as germination, fermentation and roasting (Komal and Darshen, 2000). The reduction of the tannin content of the yellow maize during fermentation may be due to microbial activity which may hydrolyse the condensed tannins to lower molecular weight phenols. Khatarpaul and Chauhan (1991) reported similar findings.

The observed reduction in the phytic acid and tannin content could also be as a result of processing which has been reported to reduce the discoloration imparted by tannin to maize (Modu *et al.,* 2012). Tobacco (2000) reported that among the processing technique, fermentation and roasting are the most effective methods in reducing the tannin contents of cereal and legumes. The result is in agrees with the findings of Modu *et al.* (2012) who reported 35 % reduction in tannin content of cereal and legumes.

Table 2: Anti-nutrients (Phytic Acid Tannin) of the Composite meal From Sorghum, Soya beans and Benni seeds (g/100g)

Samples	Phytic acid	Tannin
US	0.6600 ± 0.0176^{a}	0.5107 ± 0.0033^{a}
PS	0.5747 ± 0.0012^{b}	0.4423 ± 0.0007^{c}
FSS	0.5967 ± 0.0003^{b}	0.4733 ± 0.0019^{b}
FSSB	0.5400 ± 0.1528^{c}	0.3167 ± 0.0033^{d}

Values are expressed in Mean ±SEM, n=3. Values in the same row with different superscript are significantly different ($p<0.05$).

Key words

US: Unprocessed sorghum

PS: Processed sorghum

FSS: Fortified sorghum with soybeans

FSSB: Fortified sorghum with soybeans and benni seed

SEM: Standard Error of Mean

CONCLUSION

Therefore, this work revealed that the processing method has drastically reduced the antinutrtional factors thereby improving the nutritional quality of the composite meals. The addition of soya beans and benni seeds further furnished the protein quality of the composite meals. The benni seed also compared favourably with the soya beans in terms of the protein content. In view of this, the underutilized benni seeds should be recommended for as an alternative for protein source in food product formulations.

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