

The Potential of Sprouted Sorghum Supplemented with Sprouted Soybeans in the Formulation of Preventive Diet for Osteoporosis

Enemali Ojochenemi JohnPaul*, Omede Ameh, Eneh William Nebechukwu, Ibrahim Iye Asenetu, Ovabor Bright, Suleiman Ibrahim Zainab, Hussaini Jonathan Solomon, Igwe Chinwe Vivian, Ishaya Daniel Bwala, Rita Ifeyinwa Hanson-Akpan

National Space Research and Development Agency (NASRDA), Obasanjo Space centre, Umaru Musa Yaradua Expressway, Lugbe, Abuja, Nigeria

Abstract Osteoporosis is a disease of the bone caused majorly by vitamin D deficiency, low calcium, bad eating habits, lack of oestrogen etc. Fortified diets had been used in the treatment of clinical disorders such as this. This study investigated the effect of sprouting on formulation of preventive diets for osteoporosis from sorghum and soyabeans flour blends. The sorghum and soyabeans were sprouted separately for five days. Sprouts with maximum nutrient were obtained and milled into flours. Sorghum flour was supplemented with 0, 10, 20, 30 and 40% soyabeans respectively. Proximate analysis of the samples showed that supplementation increased the crude protein, fat, ash, crude fibre and moisture contents of the blends significantly ($p < 0.05$) from $(10.98 \pm 0.01^a - 23.01 \pm 0.01^e \%)$, $(3.28 \pm 0.01^a - 10.02 \pm 0.04^e \%)$, $(8.78 \pm 0.01^a - 10.82 \pm 0.01^e \%)$, $(1.06 \pm 0.02^a - 4.01 \pm 0.01^e \%)$, $(10.99 \pm 0.02^a - 12.72 \pm 0.01^e \%)$ respectively while carbohydrate decreased significantly ($p < 0.05$) from $(64.04 \pm 0.02^e - 39.21 \pm 0.01^a \%)$. Supplementation of 10% and 40% increased total amino acid (TAA) significantly ($p < 0.05$) from $(54.46 \pm 0.02^a - 64.45 \pm 0.01^d \text{ g}/100\text{g})$, total essential amino acid (TEAA) increased significantly ($p < 0.05$) from $(20.49 \pm 0.09^a - 26.28 \pm 0.01^e \text{ g}/100\text{g})$ same trend was observed for total non-essential amino acid (TNEAA) as it increased significantly ($p < 0.05$) from $(29.97 \pm 0.01^a - 39.19 \pm 0.04^e)$ tryptophan was the limiting essential amino acid (0.79 ± 0.01^e) while glutamic acid was the most abundant amino acid with the value (10.88 ± 0.03^e) . Generally, soya beans improved sorghum quality and food formulated from the blends may serve as a potential diet for the prevention of osteoporosis.

Keywords Osteoporosis, Phytoestrogen, Sorghum, Soyabeans, Sprouting, Vitamin D, Calcium

1. Introduction

Osteoporosis is a bone disease characterized by low bone mass and low bone mineral density (BMD), which leads to a high frequency of bone fragility and fractures [1]. The mass of skeletal bone is influenced by a number of factors majorly extrinsic (nutrition, exercise) and endogenous (genes, metabolic hormones) factors [2]. several therapeutic treatments have been designed to prevent or treat bone loss in patients suffering from osteopenia. Adequate nutrition has also been alluded as a key treatment for osteoporosis as nutrition plays an important role in bone health, and there is an increasing interest in dietary nutrients which influence bone metabolism and health such as Ca and VD [3].

Reduced dietary intake of Ca is associated with reduced

bone mass and leads to osteoporosis. Chronic VD deficiency leads to osteomalacia [4]. Therefore, maintaining adequate diet and intake of calcium and vitamin D is important in maintaining bone mass density as Vitamin D enhances intestinal absorption of calcium and phosphate. Calcium is an essential nutrient that is necessary for many functions in human health. Calcium is the most abundant mineral in the body with 99% found in teeth and bone. Research has also shown that adequate calcium intake can reduce the risk of fractures, osteoporosis, and diabetes in some populations [5].

However, Low concentrations of vitamin D are associated with impaired calcium absorption, a negative calcium balance, and a compensatory rise in parathyroid hormone (PTH), which results in excessive bone resorption [6]. Prevention of vitamin D deficiency (VDD) which in turn leads to calcium imbalance remains a health priority globally [7].

Food fortification and supplementation with Vit D is considered one of the most cost-effective strategies to combat and address VDD. Supplementation with Vit D is

* Corresponding author:

enemalij@yahoo.com (Enemali Ojochenemi JohnPaul)

Received: Jun. 7, 2022; Accepted: Jun. 27, 2022; Published: Jul. 15, 2022

Published online at <http://journal.sapub.org/food>

also effective to address VDD [7], but this requires close control of the dose due to potential toxicity, resulting in hypercalcemia and hypercalciuria [8].

Studies have equally shown that oestrogen has a protective role against osteoporosis. Soybeans have been linked to many health benefits and its rich in protein and many vitamins and minerals. It is also rich in phytoestrogens known as isoflavones. Phytoestrogens are isoflavones, which are plant-derived phenolic chemicals with estrogenic action. Soybeans and soy products are high in protein and are the richest sources of phytoestrogen compounds in human diet. In addition, soy isoflavones can produce estrogen-like activity in the body by mimicking the effects of natural estrogen [9].

Sorghum is one of the indigenous staples used in addition to other food sources in diet formulation. It is known to be rich in phenolic compounds with appreciable anti-oxidant properties. The starch from sorghum is also good in weight control, hence perfect staple for astronauts intending space mission.

Sprouting is a physical modification method used to enhance the quality of food. It is the process by which seeds or spores are induced to germinate or put out shoots mainly for the purpose of consumption or commercialization. Earlier studies have shown that sprouted foods play protective role in the body [10].

One of the major obstacles to long-term space missions is the threat of severe bone loss in astronauts. In the microgravity environment of space, astronauts lose on average 1% to 2% of their bone mineral density every month. For a short-duration flight. On a long-duration space flight, such as those planned for missions to Mars and beyond, bone loss can be a serious impediment. This loss may not hinder astronauts while they are in orbit, but upon return to Earth, their weakened bones will be fragile and at an increased risk of fractures [11].

2. Materials and Methods

2.1. Sample Collection and Preparation

Sample collection

Sorghum and Soya bean seeds were purchased from Lokoja international market Lokoja, Kogi State of Nigeria.

Sample Preparation

The seeds were hand picked to remove extraneous materials. Sorghum and soyabeans were soaked separately in water for 6 and 8 hrs to enable the seeds sprout. After soaking, the seeds were sprouted in trays covered with aluminium foils and water was sprinkled on the seeds at intervals to keep seeds wet, thereby providing an enabling environment to achieve sprouts [12]. Maximum sprouts were achieved for sorghum on day 3 while soyabeans attained full sprout on day 4 respectively. Samples were dried and milled into a fine powder using an electric mill.

2.2. Reagents and Chemicals

All chemicals and reagents used were of analytical grade produced by British Drug House (BDH) Limited, England and Sigma Aldrich Chemical Company Incorporation, Milwaukee, Wisconsin, USA.

2.3. Proximate Analysis

Proximate nutrient determination Moisture, ash, crude protein, crude fat and crude fibre were determined by standard methods [13]. Total percentage carbohydrate was determined by the difference method [14]. The method involved addition of the total values of the different parameters (crude protein, crude fat, crude fibre, moisture and ash) elements of the sample and subtracting it from 100. The value obtained was the percentage carbohydrate constituent of the sample [15].

2.4. Amino Acid Analysis

The sorghum and soyabeans' amino acid profile was analyzed using the Technicon sequential MultiSample Amino Acid Analyzer (TSM) according to the method described by [16].

2.5. Dietary Formulation

Five experimental diets were formulated using the method described by [17].

2.6. Data Analysis

Statistical Package for Social Sciences (SPSS) version 21 was used to conduct a statistical analysis on the triplicate value of the data obtained. Using the Duncan Multiple Range Test (DMRT) with a confidence level of $p < 0.05$, analysis of variance (ANOVA) was employed to determine the degree of significance.

3. Results and Discussion

Results



Sprouted sorghum

Sprouted soyabeans

Plate 1. photograph of sprouted sorghum and soyabeans

Table 1. shows the nutrient composition of sorghum supplemented with sprouted soybean flour.

The proximate composition of sprouted sorghum flour

supplemented with 0-40% sprouted soybeans flour is presented in Table 1. The results showed that supplementation of sprouted sorghum with sprouted soybeans flour increased the crude protein, fat, ash, crude fibre and moisture contents of the blends significantly ($p < 0.05$) from (10.98 ± 0.01^a - 23.01 ± 0.01^e %) for crude protein, fat (3.28 ± 0.01^a - 10.02 ± 0.04^c %) crude fibre (8.78 ± 0.01^a - 10.82 ± 0.01^e %) ash (1.06 ± 0.02^a - 4.01 ± 0.01^e %) and moisture (10.99 ± 0.02^a - 12.72 ± 0.01^e %) respectively while carbohydrate decreased significantly ($p < 0.05$) from (64.04 ± 0.02^c - 39.21 ± 0.01^a %). The enhancement in nutrient composition of the blend upon incorporation of sprouted soybeans flour into sprouted sorghum flour (Table 1) is in agreement with the reports of [17] that incorporation of sprouted soybeans to nixtamalized maize influenced the nutrient composition of the blends positively. The reason for the notable increase in the protein content obtained for the blends in the present study can be hinged on supplementation with soybeans which is a legume. This is supported by the reports of [18] that legumes are outstanding source of protein with reduced fats which makes it better options used in food fortification when compared with animal protein that is embedded with fats. In the same vein, ash, fat, fibre and moisture were increased upon supplementation. The increase in ash upon supplementation with sprouted soya beans suggests that the diet is rich in minerals, as ash is a measure of the mineral content of food. The rich mineral content in the diet will be advantageous in the maintenance of healthy bones by astronauts who go on long space mission. This is supported by the reports of [19] that nutrition is imperative in the attainment and sustenance of optimum bone mass, as well as preventing this enervating disease. The amount of ash content reported in this study for the blends is higher than that reported by [20] for sorghum diet enriched with Bambara nut. The reason for the higher value in this study is due to the richer protein content of soybeans when compared with Bambara nut. Fibre in food helps in bowel movement, prevents constipation and encourages healthy gut microbiota [21]. Consequently, Dietary fibre have been implicated to be beneficial to bone health by increasing mineral absorption and retention due to fermentation in the lower gut and solubilization of minerals [22].

The amount reported in this study suggests that the formulated diet will help in bowel movement and will enhance availability of fat-soluble vitamins when consumed. Fat in food usually contribute appreciable energy to the body and also play a very key role as carriers of fat-soluble vitamins [23]. The consistent decrease in carbohydrate upon supplementation is predictable as several findings have alluded to this trend. Bintu and other workers had reported decrease in carbohydrate while Bambara and cowpea were used to supplement some cereals. A decrease in carbohydrate was equally admitted by Baba and co-workers when ground nut was used to supplement some cereal-based food. Nevertheless, the carbohydrate content obtained in the

present study corroborates with the recommended dietary allowance (RDA) reported by [24].

The amino acid profile of sprouted sorghum supplemented with 0-40% sprouted soybean flour is presented in Table 2. The result has shown that supplementation of the sprouted sorghum with sprouted soya beans increased both the essential and non-essential amino acids significantly ($p < 0.05$). Total amino acid and total essential amino acid for 100% sprouted sorghum were (54.46 ± 0.02^a g/100g) and (20.49 ± 0.09^a g/100g) protein correspondingly.

Supplementation of 10% and 40% increased TAA significantly ($p < 0.05$) from (54.46 ± 0.02^a - 64.45 ± 0.01^d g/100g), TEAA increased significantly ($p < 0.05$) from (20.49 ± 0.09^a - 26.28 ± 0.01^e g/100g) same trend was observed for TNEAA as it increased significantly ($p < 0.05$) from (29.97 ± 0.01^a - 39.19 ± 0.04^e) tryptophan was the limiting essential amino acid (0.79 ± 0.01^c) while glutamic acid was the most abundant amino acid with the value (10.88 ± 0.03^b). Methionine, tryptophan and threonine were increased via supplementation.

The increase obtained in the total amino acid and essential amino acid is due to the supplementation with soybeans that is richer in protein and amino acids. This is in agreement with the work of [25], who reported an increase in the amino acid composition of pearl millet supplementation with soybeans protein. Furthermore, the elevated amino acid profile confirms earlier report by [26] who attained higher amino acid profile after supplementing sorghum with soybeans flour. The total amino acid reported in this study (64.45 ± 0.01^d g/100g) is in the range reported by [27] for recommended dietary allowance (RDA). Protein quality of a food sample is usually based on amino acid content of the food [28]. This translates to the essential amino acid present in the food sample. The presence of the nine standard essential amino acids in soybeans and sorghum blend obtained in the present study portrays the high quality of the formulated diet. Limiting amino acids are said to be maintenance and growth determinant because these two features are limited or regulated by the amount of essential amino acid available in the diet. The amount obtained for the limiting amino acid in this study is adequate for a standard diet.

Methionine and cysteine are two sulphur containing amino acids that are critical to the functioning of biological systems. This is because the sulphur contained in their R-group is expedient in the synthesis of macromolecules. Methionine is renowned to be the typical initiator of protein synthesis and when incorporated in proteins exhibit hydrophobic properties [29]. On the other hand, cysteine is very vital due to its ability to form disulphide bond which are responsible for the formation of protein structures and can occur in the presence and absence of enzyme interactions [30,31]. Glutamic acid which was most abundant compared to other amino acid is in conformity with the earlier findings of [32] who recorded same for diets formulated from sorghum and Bambara nut.

Table 1. Nutrient composition of sprouted sorghum flour and soya flour blend (dry matter basis)

| Blending ratios SSGF:SSBF | Parameters Crude Protein (%) | Fat (%) | Crude Fibre (%) | Carbohydrate (%) | Ash (%) | Moisture (%) |
|------------------------------|------------------------------------|-------------------------|-------------------------|-------------------------|------------------------|-------------------------|
| 100:00:00 | 10.98±0.01 ^a | 3.28±0.01 ^a | 8.78±0.01 ^a | 64.04±0.02 ^e | 1.06±0.02 ^a | 10.99±0.02 ^a |
| 90:10:00 | 12.08±0.01 ^b | 5.58±0.02 ^b | 8.92±0.01 ^b | 58.04±0.03 ^d | 2.00±0.01 ^b | 11.32±0.01 ^b |
| 80:20:00 | 16.08±0.01 ^c | 6.78±0.04 ^c | 9.02±0.01 ^c | 51.85±0.01 ^c | 2.88±0.01 ^c | 11.72±0.02 ^c |
| 70:30:00 | 19.00±0.03 ^d | 8.18±0.01 ^d | 9.92±0.01 ^d | 45.4±0.01 ^b | 3.68±0.01 ^d | 12.11±0.01 ^d |
| 60:40:00 | 23.01±0.01 ^e | 10.02±0.04 ^e | 10.82±0.01 ^e | 39.21±0.01 ^a | 4.01±0.01 ^e | 12.72±0.01 ^e |

Values are means ± SD (standard deviation) of triplicate determinations. Means on the same column with different superscripts are significantly different at $P = 0.05$. SSGF = sprouted sorghum flour; SSBF = sprouted soybean flour.

Table 2. Amino acid Analysis

| Amino acid | 100:00:00 | 90:10:00 | 80:20:00 | 70:30:00 | 60:40:00 |
|---------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Leucine | 1.21±0.01 ^a | 1.91±0.01 ^b | 1.98±0.01 ^c | 2.43±0.01 ^d | 2.51±0.01 ^e |
| Lysine | 2.61±0.01 ^a | 2.89±0.06 ^b | 3.13±0.01 ^c | 3.34±0.01 ^d | 3.57±0.03 ^e |
| Isoleucine | 4.82±0.01 ^a | 4.99±0.01 ^b | 5.13±0.01 ^c | 5.35±0.06 ^d | 5.47±0.01 ^e |
| Phenylalanine | 3.17±0.02 ^a | 3.33±0.01 ^b | 3.42±0.04 ^c | 3.58±0.01 ^d | 3.65±0.02 ^e |
| Tryptophan | 0.53±0.01 ^a | 0.58±0.03 ^b | 0.62±0.01 ^c | 0.74±0.01 ^d | 0.79±0.01 ^e |
| Valine | 2.86±0.01 ^a | 2.96±0.02 ^b | 3.12±0.01 ^c | 3.16±0.03 ^d | 3.18±0.01 ^e |
| Methionine | 0.84±0.03 ^a | 0.91±0.01 ^b | 0.93±0.03 ^c | 1.00±0.01 ^d | 1.23±0.04 ^e |
| Proline | 0.90±0.01 ^a | 1.01±0.01 ^b | 1.22±0.01 ^c | 1.52±0.01 ^d | 1.71±0.01 ^e |
| Arginine | 3.92±0.01 ^a | 4.02±0.03 ^b | 4.07±0.01 ^c | 4.10±0.02 ^d | 5.13±0.01 ^e |
| Tyrosine | 2.62±0.02 ^a | 2.69±0.01 ^b | 2.72±0.02 ^c | 2.87±0.01 ^d | 2.92±0.03 ^e |
| Histidine | 1.79±0.01 ^a | 1.91±0.01 ^b | 2.14±0.01 ^c | 2.42±0.01 ^d | 2.64±0.01 ^e |
| Cysteine | 0.95±0.01 ^a | 1.23±0.05 ^b | 1.56±0.01 ^c | 1.74±0.01 ^d | 2.99±0.02 ^e |
| Alanine | 2.29±0.01 ^a | 2.44±0.01 ^b | 2.71±0.02 ^c | 2.95±0.06 ^d | 3.14±0.01 ^e |
| Glutamic Acid | 9.06±0.02 ^a | 10.21±0.01 ^b | 10.44±0.01 ^c | 10.62±0.01 ^d | 10.88±0.03 ^e |
| Glycine | 2.39±0.01 ^a | 2.57±0.04 ^b | 2.72±0.01 ^c | 2.89±0.01 ^d | 3.15±0.01 ^e |
| Threonine | 2.75±0.02 ^a | 2.98±0.01 ^b | 3.07±0.03 ^c | 3.20±0.02 ^d | 3.29±0.02 ^e |
| Aspartic Acid | 4.88±0.01 ^a | 4.99±0.01 ^b | 5.18±0.01 ^c | 5.33±0.01 ^d | 5.72±0.01 ^e |
| Serine | 3.01±0.01 ^a | 3.21±0.02 ^b | 3.25±0.01 ^c | 3.44±0.01 ^d | 3.62±0.03 ^e |
| TAA | 54.46±0.02 ^a | 54.66±0.01 ^a | 57.43±0.02 ^b | 59.54±0.04 ^c | 64.45±0.01 ^d |
| TEAA | 20.49±0.09 ^a | 21.82±0.01 ^b | 24.46±0.01 ^c | 25.16±0.01 ^d | 26.28±0.01 ^e |
| TNEAA | 29.97±0.01 ^a | 32.28±0.03 ^b | 33.80±0.01 ^c | 35.39±0.01 ^b | 39.19±0.04 ^e |
| TEAA/TNEAA | 0.69±0.01 ^a | 0.68±0.01 ^a | 0.72±0.03 ^b | 0.72±0.02 ^b | 0.68±0.01 ^a |

Values are means ± SD (standard deviation) of duplicate determinations. Means on the same row with different superscripts are significantly different at $P = 0.05$. TAA = total amino acids. TEAA = total essential amino acid; SSGF = Sprouted sorghum flour; SSBF = sprouted soybean flour.

4. Conclusions

In this study, it was found that supplementation of sorghum with soyabeans protein enhanced the general quality of the formulated diets with increase in soyabeans content. Furthermore, the blend was found to contain the nine essential amino acids. This suggests that the formulated diet was a standard diet and be serve as a potential diet in the prevention of osteoporosis.

5. Recommendations

It is recommended that further investigations are needed on formulated diets from sorghum and soyabeans such as

phytoestrogen, antinutrients, vitamins etc in furtherance of the viability of the diets. In the same vein, other staples such as rice, millets, beans etc can be investigated. Also, the use of animal model to check toxicity, biochemical parameters, haematological indices etc are required.

ACKNOWLEDGEMENTS

The authors would like to appreciate the Laboratory Technologist at the Baze university Abuja and Federal University Lokoja for their technical support in the course of this work. Thank you most profusely.

REFERENCES

- [1] Mc-Carron DA, Heaney RP. Estimated health care savings associated with adequate dairy food intake. *American Journal of Hypertension*, 2004; 17: 88-97. <https://doi.org/10.1016/j.amjhyper.2003.08.008>.
- [2] Gennari C. Calcium and vitamin D nutrition and bone disease of the elderly. *Public Health Nutrition*. 2001; 4, Doi: 10.1079/phn2001140.
- [3] Mustafa, R. A., Alfky, N. A., Hijazi, H. H., Header, E. A., & Azzeh, F. S. (2018). Biological effect of calcium and Vitamin D dietary supplements against osteoporosis in ovariectomized rats. *Progress Nutrition*, 20 (1), 86-93. DOI: 10.23751/pn.v20i1.5223.
- [4] Palacios C. The role of nutrients in bone health, from A to Z. *Crit Rev Food Sci Nutri*. 2006; 46(8): 621- 628. <https://doi.org/10.1080/10408390500466174>.
- [5] Beto, J. A. (2015). The role of calcium in human aging. *Clinical nutrition research*, 4(1), 1-8 Doi: <https://doi.org/10.7762/cnr.2015.4.1.1>.
- [6] Rosen, H. N., Rosen, C. J., Schmader, K. E., & Mulder, J. E. (2017). Calcium and vitamin D supplementation in osteoporosis. *UpToDate, Waltham, MA. [Accès: 15 de juliol de 2014]*. <https://www.medilib.ir/uptodate/show/2023>.
- [7] Roth, D.E.; Abrams, S.A.; Aloia, J.; Bergeron, G.; Bourassa, M.W.; Brown, K.H.; Calvo, M.S.; Cashman, K.D.; Combs, G.; De-Regil, L.M.; et al. Global prevalence and disease burden of vitamin D deficiency: A roadmap for action in low- and middle-income countries. *Ann. N. Y. Acad. Sci.* 2018, 1430, 44–79. [Google Scholar] [CrossRef] [PubMed].
- [8] Haines, S.T.; Park, S.K. Vitamin D supplementation: What's known, what to do, and what's needed. *Pharmacotherapy* 2012, 32, 354–382. [Google Scholar] [CrossRef] [PubMed].
- [9] Patisaul, H. B., & Jefferson, W. (2010). The pros and cons of phytoestrogens. *Frontiers in neuroendocrinology*, 31(4), 400-419. <https://doi.org/10.1016/j.yfrne.2010.03.003>.
- [10] Nyau, V., Prakash, S., Rodrigues, J., & Farrant, J. (2017). Profiling of Phenolic Compounds in Sprouted Common Beans and Bambara Groundnuts. *Journal of Food Research*; 6, (6) 74. <https://scholar.google.com/scholar?hl=en&a>.
- [11] Suedfeld, P., Halliwell, J. E., Rank, A. D., & Buckley, N. D. (2016). Psychosocial aspects of spaceflight and aging. *REACH-Reviews in Human Space Exploration*, 2 (2), 24-29. DOI: <http://dx.doi.org/10.1016/j.reach.2016.11.001>.
- [12] James, S., Akosu, N. I., Maina, Y. C., Baba, A. I., Nwokocho, L., Amuga, S. J., ... & Omeiza, M. Y. M. (2018). Effect of addition of processed bambara nut on the functional and sensory acceptability of millet-based infant formula. *Food science & nutrition*, 6(4), 783-790. <https://doi.org/10.1002/fsn3.618>.
- [13] AOAC, Official methods of analysis of the Association of Official Analytical Chemists, 15th edn. AOAC Pub., Virginia, U.S.A; 1990. 14. <https://doi.org/10.1093/jaoac/73.1.121>.
- [14] Onyeike EN, Olungwe T, Uwakwe AA. Effect of heat treatment and defatting on the proximate composition of some Nigerian local soup thickeners. *Food Chem*. 1995; 53: 173-175. [https://doi.org/10.1016/0308-8146\(95\)90784-5](https://doi.org/10.1016/0308-8146(95)90784-5).
- [15] Etiosa, O. R., Chika, N. B., & Benedicta, A. (2017). Mineral and proximate composition of soya bean. *Asian Journal of Physical and Chemical Sciences*, 4(3), 1-6. DOI: 10.9734/AJOPACS/2017/38530.
- [16] Benitez, L.V. (1989). Amino acid and fatty acid profiles in aquaculture nutrition studies. In: S.S. De Silva (ed.). *Fish Nutrition Research in Asia. Proceedings of the Third Asia Fish Nutrition Network meeting*. Asian Fish Society Special Publication, 4, 166. DOI: 10.12691/ajfn-4-6-2.
- [17] Inyang, U. E., Akindolu, B. E., & Elijah, A. I. (2019). Nutrient composition, amino acid profile and anti-nutritional factors of nixtamalized maize flour supplemented with sprouted soybean flour DOI: 10.9734/EJNFS/2019/46150.
- [18] Maphosa, Y., & Jideani, V. A. (2017). The role of legumes in human nutrition. *Functional Food-Improve Health Through Adequate Food*, 1, 13. <http://dx.doi.org/10.5772/intechopen.69124>.
- [19] Sahni, S., Mangano, K. M., McLean, R. R., Hannan, M. T., & Kiel, D. P. (2015). Dietary approaches for bone health: lessons from the Framingham Osteoporosis Study. *Current osteoporosis reports*, 13(4), 245-255. <https://link.springer.com/article/10.1007/s11914-015-0272-1>.
- [20] Baba, G. M., Modus, S., Falmata, A. S., Hajjagannah, L., & Ibrahim, Z. (2012). Evaluation of the nutritional value of sprouted sorghum fortified with cowpea and groundnut. *Journal of Agricultural Science*, 2 (11), 292-296: <https://scho.larly-journals.com/sjas/archive/2012/nov/pdf/Baba%20et%20al.pdf>.
- [21] Fuller, S., Beck, E., Salman, H., & Tapsell, L. (2016). New horizons for the study of dietary fiber and health: a review. *Plant foods for human nutrition*, 71(1), 1-12. <https://link.springer.com/article/10.1007/s11130-016-0529-6>.
- [22] Weaver, C. M., Martin, B. R., Story, J. A., Hutchinson, I., & Sanders, L. (2010). Novel fibers increase bone calcium content and strength beyond efficiency of large intestine fermentation. *Journal of agricultural and food chemistry*, 58(16), 8952-8957. <https://doi.org/10.1021/jf904086d>.
- [23] Ihekoronye AI, Ngoddy PO. Integrated food science and technology for the tropics. Macmillan Edu. Publishers, London. 1985; 243-239: 283-292 <https://www.cabdirect.org/cabdirect/abstract/19860409222>.
- [24] Bintu B. P., Falmata A. S., Maryam, B. K., Zainab, M. A. & Modu, S. (2017) Effect of feeding complementary diet blends formulated from *Zeamays* (maize), *Vigna unguiculata* (cowpea), *Vigna subterranean* (Bambara nuts) *Arachis hypogea* (groundnut) on weaning rats. *African Journal of Food Science and Technology*, 8 (5), 099-107. DOI: <http://dx.doi.org/10.14303/ajfst.2017.114>.
- [25] Ahmed, I. A. M., El Tinay, A. H., & Babiker, E. E. (2009). Supplementation of pearl millet with soybean: Changes in protein digestibility, amino acid composition and sensory quality during processing <https://www.researchgate.net/profile/Isam-Mohamed-Ahmed/publication/228506429>.
- [26] Awadalkareem, A. M., Mustafa, A. I., & El Tinay, A. H. (2008). Protein, mineral content and amino acid profile of sorghum flour as influenced by soybean protein concentrate supplementation. *Pakistan Journal of Nutrition*, 7(3), 475-479. <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.321.507&rep=rep1&type=pdf>.

- [27] Ijarotimi, O. S., & Keshinro, O. O. (2013). Determination of nutrient composition and protein quality of potential complementary foods formulated from the combination of fermented popcorn, African locust and Bambara groundnut seed flour. *Polish Journal of Food and Nutrition Sciences*, 63(3), 155-166 DOI: <https://doi.org/10.2478/v10222-012-0079-z>.
- [28] Singer, W. M., Zhang, B., Mian, M. R., & Huang, H. (2019). Soybean amino acids in health, genetics, and evaluation. In *Soybean for human consumption and animal feed* (p. 14). IntechOpen. DOI: 10.5772/intechopen.89497.
- [29] Ingenbleek Y, Kimura H. Nutritional essentiality of sulphur in health and disease. *Nutrition Reviews*. 2013; 71(7): 413-432 DOI: 10.1111/nure.12050.
- [30] Brosnan JT, Brosnan ME. The sulfur-containing amino acids: An overview. *The Journal of Nutrition*. 2006; 136(6): 1636S-1640S DOI: 10.1093/jn/136.6.1636s.
- [31] Fontecave M, Atta M, Mulliez E. S-adenosylmethionine: Nothing goes to waste. *Trends in Biochemical Sciences*. 2004; 29(5): 243-249 doi: 10.1016/j.tibs.2004.03.007.
- [32] Sulieman, A. E. (2016) Chemical composition and in vitro protein digestibility of hulled and de-hulled Bambara groundnut] *Vigna subterranea* (L.) Verdc. Seedsfile:///C:/Users/USER/Downloads/Bambaragroundnutpaper2%20(1).pdf.