

Chemical, *In-vitro* Multienzyme Digestibility and Amino Acid of Sorghum (*Sorghum bicolor* (L) Moench) Fortified with Soybean (*Glycine max*) Composite Meal flour

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The proximate, minerals, functional properties, in-vitro multi enzyme protein digestibility and amino acid of sorghum fortified with soybean composite meal flour have been determined using standard analytical procedures. The sample contained moisture (3.52%), ash (3.64%), crude fat (27.44%), crude protein (39.33%), crude fibre (5.66%) and carbohydrate (20.41%). The magnesium was the highest mineral with the value of 40.78mg/kg while copper had the lowest value of 0.04 mg/kg. The results of functional properties showed that water absorption capacity (WAC) had the value of 142.62%, oil absorption capacity (OAC) had the value of 114.24%, foaming capacity/stability (6.50%/2.00%) while the least gelation concentration was 2.00%. The multi enzyme protein digestibility was 66%. Glutamic acid was the most concentrated amino acid with the value of 172.5 mg/g crude protein while cysteine was the least concentrated with the value of 20.5 mg/g crude

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protein. The average total amino acid in the composite sample was 915.5 mg/g crude protein and percent total essential amino acid was 44.7% (with histidine), 42.3% (without histidine). The composite sample meal flour would provide the required amino acids for adults and pre-school children (2-5yrs) since the score values obtained were higher than 100% required standards recommended for the various groups.

Keywords: Chemical; in-vitro multienzyme digestibility; amino acid; fortified; sorghum; soybean; composite.

1. INTRODUCTION

“Plant resources are used predominantly by the developed and underdeveloped countries to circumvent hunger and food insecurity. The foods for human consumption are plants mostly consumed by rural dwellers and peasant farmers. It is well understood that the developing countries do not produce enough food with correct nutritional quality to cater for daily needs of the citizens” [1]. “The nutritional status of the population plays a key role in human resource development of a country. Human resource development is enhanced by nutrition improvement” [2]. In Nigeria, plant sources are the cheapest and reliable means of protein intakes. Cereal grains are the most staple food for the people of tropical and sub-tropical parts of the world. Cereals are plant based diets contributing the highest human’s calorific intakes. The survival of the people in arid region could be attributed to the consumption of cereals. Ihekoronye and Nggody [3] reported that most cereals contain vitamins, minerals and essential amino acids with limited lysine and sulphur containing amino acids that are required by man particularly when supplemented with other foods. Eka [4] reported that cereal-based weaning porridges are of nutritive value and have been implicated in the incidence of protein-energy malnutrition, a major cause of high infant mortality. The improvement of the nutritive value of cereal-based foods has been achieved by incorporation of grain legumes [5-8].

Sorghum (*Sorghum bicolor* (L) Moench) probably originated from tropical Africa. It forms the staple crop of the people dwelling in the Northern parts of West Africa. Sorghum germinates well where rainfall is between 380-640mm while growing and dry hot weather while ripening. It grows well on a variety of soils with pH of 5.5-8.5 except sand and clay while a temperature of 28^oC is required for maximum yields. Sorghum is eco-friendly cereal crop with tremendous and promising food properties. It has low protein and moderately high in carbohydrate. This factor

makes it a substitute for wheat in bakery and brewing industries as well as in the preparation of local wine called “Burukutu” in Yoruba tribe of Nigeria; pap and additive for local milk drink called “Fura de nunu” in Hausa tribe of Nigeria. “It is a multipurpose minor cereal crop primarily used as food, feed, forage, most importantly as raw material for brewing liquor [9,10] and value-added food products” [11]. “It is also widely grown on dry-land agricultural systems in arid and semiarid specific zones of northern and northeastern parts of China. In these areas, the excessive consumption of water and over application of inorganic fertilizers have led to serious environmental problems for sustainable agriculture” [12]. “These characteristics limit the productivity and make dry-land agro-ecosystems both inherently dynamic and vulnerable” [10]. “From ethnomedicinal view, sorghum has some health potentials due to its high oxidant power as anti-inflammatory, anti-proliferative, anti-diabetic and anti-atherogenic” [13]. “Its phenolic compounds can prevent many diseases including cancer, diabetes, digestive tract and cardiovascular diseases” [14,15].

Soybean (*Glycine max*) is annual herbaceous specie that grows to roughly 1 meter in height and may be branched [16]. It is prevalently grown and used as oilseeds. “It uses ranged from human foods to animal foods to industry” [17]. Oluwagbenle [18] reported that “soybean seeds can be processed to give soy-milk, which is an excellent source of protein for babies especially those just weaned from breast feeding and the invalids”. The seed has multidimensional values that transcend from food industry to pharmaceutical industry. Soybean has exceptionally high oil and protein content among legume family and is often converted into various food products by fermentation with the aid of moulds or bacteria to improve their flavor as well as nutritive value. In Nigeria, soybean can be locally and industrially extracted as well as formulated to a recipe known as “soy-ogi”- a protein formula normally used as a weaning food for the infants, malnourished children and adults.

The formulation involved the wet milling of steeped sorghum and soybean into slurry of which is allowed to ferment. The fermented sorghum–soy mash may be fortified with minerals, vitamins, colourants, pasteurized and then spray-dry into flour followed by packaging.

Oluwagbenle [18] studied the nutritional evaluation of pearl millet fortified with soybean flour. It was observed that the food properties and qualities of the cereal (pearl millet) were consequently improved when fortified with soybean. Therefore, the purpose of this study is to know whether the formulation of sorghum – soybean composite through fortification process would enhance and improve the nutrients density, nutritional potential and quality of sorghum-soybean meal flour.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Sorghum (reddish brown) and soybean (white) grains were purchased from the main market in Akure, Ondo State, South west of Nigeria. The two samples were thoroughly screened to remove the bad ones and the remaining good ones were dry-milled into powder using Kenwood blender, sieved using 0.5mm mesh size sieve.

Equal portions of sorghum and soybean in ratio 50:50 were thoroughly mixed by quartering method to obtain the sorghum-soybean fortified composite meal flour sample. The representative composite fortified sample obtained was then packed into a sample glass bottle and stored in a freezer prior to analyses.

2.2 Determination of Proximate Composition

The moisture was analyzed using air-oven at temperature of 105°C for 1 hour while the ash content was determined using a muffle furnace at 550°C for 6 hours [19]. The sample was analyzed for crude fat and crude protein according to the methods described by AOAC [20]. The crude fibre was determined by adding 2g of the sample into 500cm³ conical flask; 200 cm³ of boiling 1.25% H₂SO₄ was added and boiled for 30 minutes. The mixture was filtered through muslin cloth and rinsed with hot distilled water. The sample was scrapped back into the flask and 200 cm³ of boiling 1.25% NaOH was added and allowed to boil again for another 30 minutes; filtered and then rinsed with 10% HCl

twice with industrial methylated spirit, drained and dried. The residue was scrapped into crucible, dried in the oven at 105°C and then allowed to cool in the desiccator and weighed; later placed in the muffle furnace at 300°C for 30 minutes and then finally allowed to cool at room temperature and re-weighed” [19]. The total carbohydrate was obtained by method of difference.

$$\text{CHO} = 100 - [\% \text{ Moisture} + \% \text{ Fat} + \% \text{ Ash} + \% \text{ Crude Fibre} + \% \text{ Crude Protein}] \quad (1)$$

2.3 Determination of Mineral Composition

The composite sample (1.2g) was weighed into a crucible and placed in a muffle furnace at a temperature of 550°C for 6 hours to obtain a complete ash without black specks. The ash was digested with 3cm³ of 3M HCl and made up to the mark with 0.36M HCl in a 100 cm³ standard flask with deionized water. “The mineral analysis was performed using Atomic Absorption Spectrophotometer (Buck Scientific Model-200 A/210, Norwalk) and phosphorus was determined colorimetrically by Spectronic 20 (Gallenpkam, UK) [20]”.

2.4 Determination of Functional Properties

“The water and oil absorption capacities of the sample were determined using the method of Beuchat” [21]. A 10 cm³ of water was added to 1.0g sample in a centrifuge tube. The suspension was mixed vigorously using Vortex mixer. This was then centrifuged at 15,000 rpm for 15 minutes and the volume of supernatant left after centrifuging was noted. Water bound was calculated from the difference in the initial volume of the solvent used and final volume after centrifuging. The same procedure was used for oil absorption capacity by replacing water with oil in the above process. Emulsion was prepared according to methods of Lin et. al. [22] and Sathe and Salunkhe [23]. Sample flour (2g) was weighed with 100 cm³ distilled water and blended for 30 seconds using Kenwood food mixer at high speed. After complete dispersion, vegetable oil of density 0.880g per cm³ was added to 5 cm³ portion from a burette with continuous blending until the emulsion break point (i.e. a separation into two layers) was observed. Emulsion capacity and stability determinations were carried out at 25°C and the values obtained were expressed as gram of oil emulsified by 1 gram sample. The emulsion stability was determined as the amount

of the water separated after 24 hours at room temperature [24]. The slight modified procedure of Sathe et. al. [25] was used to determine the least gelation concentration. Sample slurries range of 2 – 20% w/v was prepared in 5 cm³ of distilled water. The test tubes containing these slurries were heated for one hour in boiling water followed by rapid cooling for 2 hours at 4°C. The least gelation concentration was determined as the concentration which did not slip when the test tubes were inverted. The method of Coffman and Garcia [26] was employed to determine foaming capacity and stability. One gram of the sample was whipped with 50 cm³ distilled water for 5 minutes in a Kenwood blender and later poured into a 100 cm³ graduated flask to measure the foaming capacity/stability [24].

2.5 Determination of Protein Solubility

The protein solubility was determined using the method described by Ige et al. [27]. Composite flour (0.2 g) was thoroughly mixed with 10 cm³ of distilled water using a magnetic stirrer at room temperature. The pH of the slurries prepared from samples was adjusted to values ranged between 1 and 12 using either 0.1M HCl and /or 0.1M NaOH. The mixture was centrifuged at 3,500 rpm for 30 minutes to remove the insoluble matters. The supernatant was digested and the nitrogen content determined by the Biuret method [28]. The percentage nitrogen was converted to crude protein by multiplying 6.25.

2.6 Determination of *In-vitro* Multi Enzyme Protein Digestibility

The determination of *in-vitro* protein digestibility was carried out using the method of Hsu et al. [28]. The sample suspension was prepared by dissolving 1.75g of the sample in 50 cm³ of distilled water and adjusted to pH of 8.0 using either 0.1M HCl and/ or 0.1M NaOH while stirring in a water bath maintained at 37°C. The multi enzyme solution consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg of peptidase per ml was maintained in an ice bath and adjusted to pH 8.0 (with 0.1M HCl and /or 0.1M NaOH). A 5 cm³ of the multi enzyme solution was then added to the suspension with constant stirring at 37°C. The pH of the suspension was recorded at 10 min and 15 min respectively just immediately after the addition of the multi enzyme solution and the *in-vitro* protein digestibility was calculated using the regression equation of Hsu et al. [29] as follows:

$$Y = 210.46 - 18.10 x \quad (2)$$

Where Y is the *in-vitro* multi enzyme protein digestibility (%); and x is the pH of sample suspension after 10 min or 15 min digestion with the multi enzyme solution.

2.7 Determination of Amino Acid

The amino acid profile was determined using the method described by Spackman et. al. [30]. "The sample was dried to constant weight and then defatted using Soxhlet extractor. After the defatting process, the defatted sample (2g) was weighed into a glass ampoule; 7 cm³ of 6M HCl was added and oxygen was expelled by passing through nitrogen into the glass ampoule sealed with Bunsen burner flame and placed in an oven preset at 105±5°C for 22 hours. The ampoule was allowed to cool before broken at the tip and the content was filtered to remove the organic matters. The filtrate was then evaporated to dryness at 40°C in a rotary evaporator. The residue was dissolved in 5cm³ of acetate buffer (pH 2.0), stored in a specimen bottle and kept in the freezer. The hydrolysate (7.5µL) was dispensed into the cartridge of the Technicon Sequential Multi-Analyzer (TSM) using a syringe. The TSM analyzer is designed to separate and analyze neutral, acidic and basic amino acids of the hydrolysate. The amount of amino acids was obtained from the chromatogram peaks. The whole analysis lasted for 76 hours and the gas flow rate was 0.50 cm³ per minute at 60°C with reproducibility consistent within ±3%" [24].

Method of calculating amino acid value from the chromatogram peaks.

The net height of each peak produced by the chart record of the TSM (each representing an amino acid) was measured. The half-height of the peak at the base was also measured. The approximate area of each peak was then obtained by multiplying the height with the width at half-height.

The Norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using equation [3].

$$NE = \frac{\text{Area of Norleucine peak}}{\text{Area of each amino acid}} \quad (3)$$

A constant S was calculated for each amino acid in the standard mixture:

$$S_{\text{std}} = NE_{\text{std}} \times \text{Mol. Weight} \times \mu\text{MAA}_{\text{std}} \quad (4)$$

Finally, the amount of each amino acid present in the sample was calculated in g/100g crude protein using equation (4):

$$\text{Concentration (g/100g crude protein)} = \frac{\text{NH} \times \text{NH}/2 \times \text{S}_{\text{std}} \times \text{C}}{\text{NH} \times \text{W} \times (\text{N} - \text{leu})} \quad (5)$$

Where C =

$$\left(\frac{\text{Dilution} \times 16}{\text{Sample wt. (g)} \times \text{N2\%} \times 10 \times \text{Vol. loaded}} \right) \div \text{NH} \times \text{W} \times (\text{N} - \text{leu}) \quad (6)$$

And

NH = Net Weight

W = Width

N-leu = Norleucine

2.8 Estimation of Quality of Dietary Protein

The quality of dietary protein can be in various ways [31], but basically, it is the ratio of available amino acid in the food or diet compared with needs expressed as ratio [32-34]. Equation 7 was used to calculate the essential amino acid score:

$$\text{Amino acid score} = \frac{\text{mg of amino acid per of test protein}}{\text{mg of amino acid per g protein in reference pattern}} \quad (7)$$

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of Sorghum Fortified with Soybean Composite Flour

The chemical composition of food derived from plants is dependent on: variety, hybrid, geographic location and growth conditions. The sorghum fortified with soybean has a low moisture content (3.52%), this is an added advantage as the quality is enhanced for a long time because, low moisture content prevents microbial spoilage and pest attack during storage. The moisture content was in close agreement with those obtained for gourd seeds (3.46%) [35], for melon seeds (5.0%) [36], fluted pumpkin (5.0%) [37] but relatively lower than those of water melon [38] and soybean (4.12%) [39]. The average value of ash content of the sorghum fortified with soybean sample was 3.64%. The composite sample had low value of total ash. Therefore, it precludes a reasonably high quality of minerals.

Table 1. Proximate composition (%) of sorghum fortified with soybean composite flour

Proximate	Composition (%)
Moisture	3.52
Total ash	3.64
Crude fat	27.44
Crude protein	39.33
Crude fibre	5.66
Carbohydrate	20.41

The fat content was 27.44% which was lower than 30.8% for *Brachystegia eurycoma* seed [40] and also higher than values reported for *Cactus per cladades* (2.30%) [41], pigeon pea (6.49 – 6.57%) [42] and (6.96%) *Caesalpinia pulcherrima* [43] respectively. The crude fat value of sorghum fortified with soybean composite flour obtained in this study was far higher than that obtained for sorghum in the range (2.10-7.60%) [44]. It showed that there was an improvement/enhancement over the unfortified sorghum. The crude fat content does not qualify unfortified sorghum as oil rich crop when compared with groundnuts [45] but fortification makes it richer in oil. Food legumes in general have higher concentration of fat than cereals [45]. The mean value for the crude protein of the sorghum fortified with soybean composite sample was 39.33%. The use of protein rich source like soybean together with cereal like sorghum enables the fortified product a potential protein source of food for both man and livestock. It has also been shown that legume-based fortified weaning foods are of good nutritive value and have shown to prevent protein-energy malnutrition [7,8,46,47]. The crude protein (39.33%) was found to be comparably higher than some other leguminous seed flour such as *Brachystegia eurycoma* (35.8%) [40], *Sphenostylis sternocarpa* (18.55%) [48], African yam bean varieties [49], cucumber peel (26.5%), pulp (15.9%) and seed (24.5%) reported by Oluwagbenle et al. (2019). This value was also higher than that obtained for unfortified sorghum flour (15.9%) [50]. Crude fibre also known as roughage consists mainly cellulose and hemicellulose (heterogenous group) in which pentosan usually predominate over lignin, pectic and cutin substances (Salunkhe et al. 1985). The average crude fibre for sorghum fortified with soybean composite sample was 5.66%. Carbohydrates from human diets are primarily derived from plants especially, cereals, tubers etc. Carbohydrates supply major portion of man's energy. The mean value

obtained for carbohydrate in sorghum fortified with soybean composite sample was 20.41%. This showed that sorghum fortified with soybean had very low carbohydrate content due to enhancement after fortification process which in turn changed the narration of sorghum as carbohydrate-based food to protein-rich food. The carbohydrate value was lower than that obtained for bread produced from indigenous AC3B yeast isolate (43.33%) [51].

3.2 Mineral Composition of Sorghum Fortified with Soybean Composite Sample

The values for mineral composition of sorghum fortified with soybean composite flour are shown in Table 2. Minerals such as Ca, Mg, Na, K, Fe, Mn, Zn, Cu and Pb were determined. All these minerals are essential minerals. The sample was found to be richer in magnesium (40.78 mg/kg), sodium (22.94 mg/kg), potassium (25.51 mg/kg), Calcium (7.17 mg/kg), Zinc (2.34 mg/g) and Iron (0.49 mg/kg). Sodium is useful for intercellular and intracellular transport systems in the body fluid. The composite sample contained moderate amount of sodium and potassium. Magnesium was the most abundant mineral while palladium and copper were found to be the least.

Table 2. Mineral composition (mg/kg) of sorghum fortified with soybean composite flour

Mineral	mg/kg
Calcium	7.17
Magnesium	40.78
Sodium	29.94
Potassium	25.51
Iron	0.49
Manganese	0.08
Zinc	2.34
Copper	0.04
Palladium	0.04
Na/K	1.17
Ca/Mg	0.18
[K/(Ca+Mg)]	0.53

N.D = Not detected

Magnesium forms part of the skeleton and is an activator of various enzymes. The concentration of calcium in the composite sample amounted to 7.17 mg/kg. Calcium is an essential component as it helps in bone formation. Iron is another essential mineral which amounted to 0.49mg/mg. This element is essential for the formation of red blood cells most importantly haemoglobin [1] and some enzymes. Zinc is another essential mineral

which had a concentration of 2.34 mg/kg. The contents of Manganese (0.08 mg/kg), Copper (0.04 mg/kg) and Palladium (0.04 mg/kg) were very low. The low value of copper may be due to infinitesimal uptake /absorption of Cu^{2+} ions from the soil by the plant through translocation process. The copper (II) ions might probably have been leached into the soil from the intentional application of herbicides to control weeds or chemicals containing CuSO_4 to exterminate possible pathogens and fungi affecting plants on farmland. Iron is important in the formation of blood in the body. The level of iron in the composite sample studied was found to be higher than that of quinoa flour reported by Ogungbenle [1]. The value of Ca/Mg was calculated to be 0.18, this value was less than the minimum level recommended by NRC [52]. The calculated value of 0.53 obtained for $[\text{K}/(\text{Ca}+\text{Mg})]$ milliequivalent was far less than 2.2. It has been recommended that the value of $[\text{K}/(\text{Ca}+\text{Mg})]$ must be less than 2.2 to prevent hypomagnesemia [53,54]. Therefore, sorghum fortified with soybean would prevent hypomagnesemia in both children and adults if consumed. The calculated value of Na/K was slightly higher than 0.60 recommended for high blood pressure patients [55]. The value obtained in the present study makes it still safe for onset hypertensive patients if consumed. In general, the mineral composition of sorghum-soybean composite flour was higher than those reported for different varieties of whole sorghum grains [10]. This trend is definitely envisaged due to improvement upon fortification of sorghum by soybean in this study.

3.3 Functional Composition of Sorghum Fortified with Soybean Composite Sample

“The functional properties are the intrinsic physico-chemical properties which describe how a protein will behave in a food system. Properties such as solubility, viscosity, texture, water and fat binding, emulsion, foam and gel” [56]. Table 3 shows the values of the functional properties of the composite sample. The value of water absorption capacity (WAC) of the sorghum fortified with soybean was 142.62 %. WAC is the ability of the composite flour to retain and maintain water against gravity and is improved by supporting hydrophilic parameters like polar groups and charged side chains [57]. This value obtained for WAC was found to be higher than those of different sorghum that ranged from 103.43 to 132.86% [10], quinoa flour (147%) [1],

pearl millet fortified with soybean flour (148.1%) [18], water leaf (137.5%) [37] and protein concentrate of sesame flour (25.7%) [58], this indicates that it can absorb water more than the compared samples. The WAC of sorghum fortified with soybean was moderately high which shows that the sample has good property of protein in viscous food like gravies, doughs, baked products e. t. c. Hence, may be useful in food formulation. The improvement was due to fortification of sorghum flour with the incorporation of soybean flour. The function of oil absorption capacity (OAC) in food industry is the interaction between the non-polar amino acid side chains and hydrocarbon chains of lipid to evaluate the flavor and mouth feel relation ability of the products [59]. The OAC of composite sample was 114.24%. The value of OAC was found to be higher than those values obtained for water leaf (32.4%) [37], benniseed (45.5%) [60], cowpea (46.0%), soy flour (84.4%), wheat flour (84.2%) [22], sorghum (55.0%) [61] and pigeon pea flour (45.70%) [62] but lower than those of the *Celosia spicata* [63], dried Roselle flower calyx (496%) reported by Oluwagbenle [24], quinoa flour (147.0%) [1] and pearl millet fortified with soybean (148.1%) [18].

Table 3. Functional composition (%) of sorghum fortified with soybean composite flour

Functional properties	Value
Water Absorption Capacity (%)	142.62
Oil Absorption Capacity (%)	114.24
Foaming Capacity (%)	6.50
Foaming Stability (%)	2.00
Emulsion Capacity (%)	47.90
Emulsion stability (%)	50.00
Least Gelation Concentration (%W/V)	2.00
Bulk density (g/cm ³)	59.25

The results of the foaming capacity and foaming stability for sorghum fortified with soybean were 6.50% and 2.00%. These values were lower than those of benniseed foaming capacity of 18.0% [60], quinoa (9.0%) [1], soy flour (16.0%, 14.6%), pigeon pea (68%, 20%) [62], *Habiscus sabdariffa* calyx (16%) as reported by Oluwagbenle [24] and pearl millet fortified with soybean (11.30%) reported by Oluwagbenle [18], this indicates that sorghum fortified with soybean has low foaming capacity. The value of emulsion capacity (47.90%) of the composite sample was higher than those of *Habiscus sabdariffa* calyx (5.0%) [24] and benniseed (30.0%) [1], this shows that the composite sample may be useful in binder formulation and colloidal foods. It may also

indicate that the composite flour may be good substitute for some legumes as food additive. The emulsion stability was 50.00%. This implies that the composite sample has high emulsion stability and may be good in quality food formulation for adults and children. The value for the least gelation concentration of composite sample (2%) was lower than that of benniseed (18.0%) [60]. The ability of this composite sample to form gels and provide a structured matrix for holding water, flavours, sugars and food ingredients is useful in food application and in new products development, thereby providing an added dimension to food functionality [64]. The low gelation concentration observed may be an asset in the use of the composite flour sample for formulation or as an additive to other gel-foaming materials in food products [65]. The result of the lowest gelation of sorghum fortified with soybean (2%) shows that it has a very good gelation and this makes it very useful for gel formation in food products. The bulk density of the composite sample was 59.25 g/cm³. The dependence of pH on protein solubility of the fortified sorghum with soybean composite flour is depicted in Fig. 1. The solubility of proteins is the manifestation of the equilibrium between protein – solvent and protein – protein interactions [66]. It is worth noting that sorghum fortified with soybean composite flour was high at both acid and alkaline media. For the composite sample studied, the minimum solubility was at pH 4.0 and maximum at pH 11. Fig. 1 also indicated the minimum protein solubility at pH 4.0 which corresponds to isoelectric point of the protein since proteins are least soluble at their isoelectric points. The pH value of minimum protein solubility was observed to be lower than those of pearl millet (pH 6.0) [60], pigeon pea (pH 5.0) [62] and fluted pumpkin seed (pH 4.0) [37] but slightly higher than that of pearl millet fortified with soybean (pH 3.0) [18]. Therefore, the moderately low solubility of the protein of sorghum fortified with soybean in the acid region of pH implies that the protein may be useful in formulation of both carbonated beverages [67] and low-acid foods [1].

The plot of pH against time is the in-vitro protein multi enzyme digestibility of sorghum fortified with soybean composite flour as depicted in Fig. 2. The average digestibility value of the composite sample flour was 60%. The value of 60% obtained was found to be lower than those values reported for dehulled African nutmeg (78.44%) [68], pigeon pea (77%) and heat –treated pigeon pea

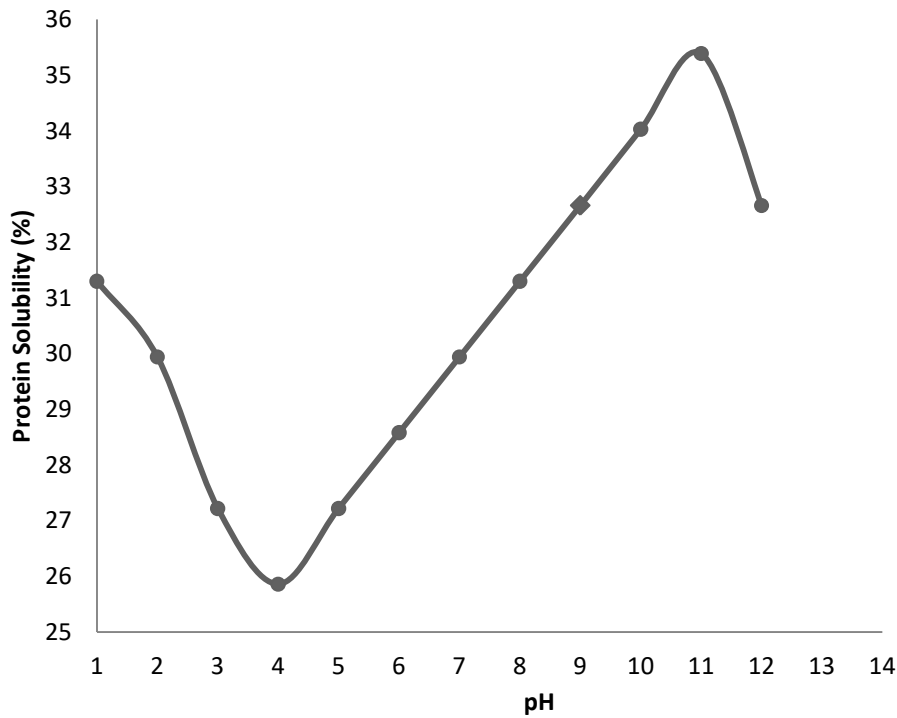


Fig. 1. Protein solubility profile of the composite sample flour

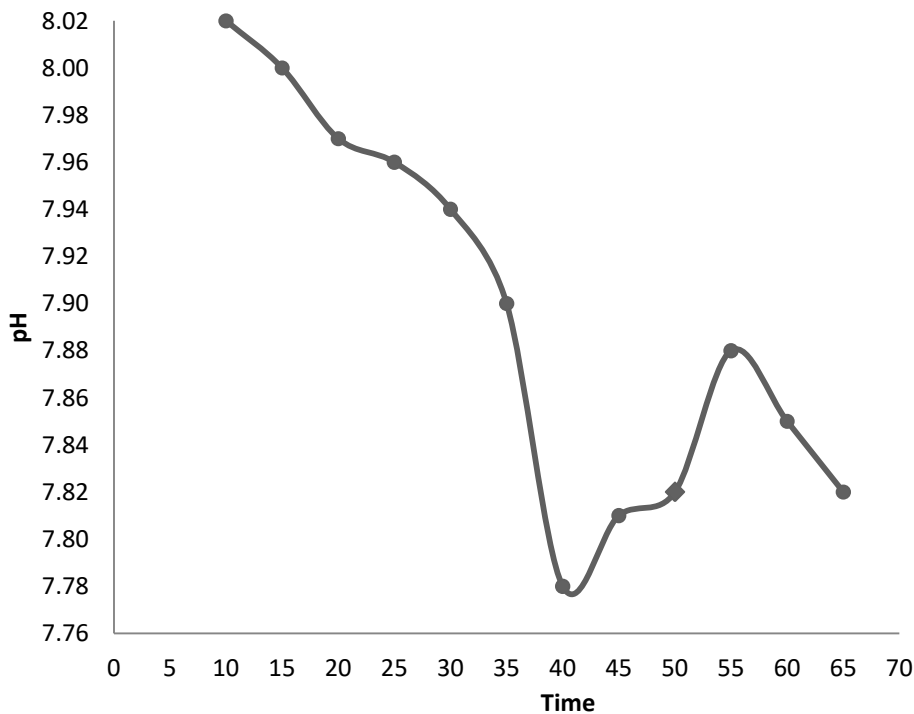


Fig. 2. In - Vitro protein digestibility of sorghum fortified with soybean flour

(84%) [69] and *Azelia africana* (77.5%) [70]. From Fig. 2, it can be explained that the pH dropped rapidly as the time increased during the hydrolysis. The protein digestibility increased as

pH decreased similar to that reported by Ogungbenle and Ebadan [57] for sesame seed protein concentrate. The trend in the graph showed that the pH dropped rapidly at the

beginning and then formed a dip after 40 minutes (pH=7.78) (minimum digestibility) and started rising to a maximum at 55 minutes (pH=7.88 (maximum digestibility).

The amino acid profile of sorghum fortified with soybean composite sample is shown in Table 4. Glutamic acid was the most concentrated amino acid in the sample with the value of 172.5 mg/g crude protein, aspartic acid occupied the second position while methionine was found to be least amino acid with the value of 21.4 mg/g crude protein. It was observed that the present result was similar to those reported for oil seeds [71] and pearl millet fortified with soybean [24] where aspartic and glutamic acids were the major abundant amino acids. The average value of isoleucine in the sample was 39.9 mg/g crude protein. Isoleucine is an essential amino acid (EAA) for both adults and children. Maple syrup urine disease (MSUD) is an inborn aberration of metabolism of some amino acids that result to brain damage and early death which can be avoided by a diet containing adequate isoleucine and two other essential amino acids (leucine and valine). Methionine is an essential amino acid with the average value of 21.4mg/g in the composite sample. Methionine is needed for the synthesis of choline. Choline forms lecithin and other phospholipids in the body. When diet is low in protein, for example, drinking alcohol significantly increases choline metabolism since alcoholics require more choline and may be deficient in choline and therefore, result to liver cirrhosis as well as kwashiorkor due to insufficient choline and accumulation of fat in the liver called fatty liver disease (hepatic steatosis).

Many parameters are depicted in Table 5. The value of total amino acid (TAA) in the composite sample was 915.5 mg/g crude protein and found to be within the range reported for dehulled African yam bean (AYB) (702.86 – 917.48 mg/g crude protein) [72] and pearl millet fortified with soybean (842.8 mg/g) [18]. The total non-essential amino acid (TNEAA) was 506.3 mg/g while that of total essential amino acid (TEAA) was 409.2 mg/g with histidine and without histidine (386.89 mg/g). Tryptophan was not determined in the sample. This result was slightly lower than the total essential amino acid (TEAA) value of cow's milk (490 mg/g) with histidine but no tryptophan and 433 mg/g (without histidine, no tryptophan), and egg (495 mg/g) with histidine, no tryptophan, and 473 mg/g (histidine, no tryptophan) [73]. The average percent TNEAA in the composite sample was 55.3% while the

average percent TEAA was 44.7% (with histidine) and 42.3 % (without histidine). It indicates that the composite sample would not be a good source of TEAA for children. The TNEAA (506.3mg/g) was found to be higher than that of the soybean (444 mg/g) [74].

Table 4. Amino acid composition

Amino acid	Concentration (mg/g crude protein)
Lysine (Lys)*	37.5
Histidine (His)*	22.4
Arginine (Arg)*	51.7
Aspartic acid (Asp)	78.0
Threonine (Thr)*	39.5
Serine (Ser)	38.6
Glutamic acid (Glu)	172.5
Proline (Pro)	62.1
Glycine (Gly)	35.6
Alanine (Ala)	65.0
Cysteine (Cys)	20.5
Valine (Val)*	52.3
Methionine (Met)*	21.4
Isoleucine (Ileu)*	39.9
Leucine (Leu)*	96.5
Tyrosine (Tyr)	34.0
Phenylalanine (Phe)	48.0

* Essential amino acids

The percentage of total neutral amino acid (% TNAAs) in the composite sample was 60.5. The high value of %TNAAs may indicate that TNAAs forms bulk of the amino acid. The total percentage of neutral amino acid (% TNAAs) was 60.5, %TNEAA was 55.3, %TEAA with histidine was 44.7. The % TAAAs was 27.4 while that of % TBAAAs was 12.2. The overall trend according to Table 5 is %TNAAs>%TNEAA>%TEAA>%TAAAs>%TBAAAs>%TSAAAs.

Table 6 showed that lysine was the limiting amino acid based on the score while isoleucine and threonine had similar score of 0.99. Valine had a score value of 1.05, methionine + cysteine had a score value of 1.20, phenylalanine + tryptophan had a score value of 1.37 while leucine recorded a score value of 1.38 respectively. It has been shown that both histidine and arginine are particularly essential for children [73, 75, 76] and the values obtained show that sorghum fortified with soybean is a good source of amino acids and would supply adequate essential amino acids for pre-school children between the ages of 2 to 5 years, since the scores were above 100% except for isoleucine and threonine (99%) that

Table 5. Essential, non essential, neutral, acidic and basic amino acids (mg/g crude protein)

Amino acid	mg/g crude protein
Total amino acid (TAA)	915.5
Total non essential amino acid (TNEAA)	506.3
Total essential amino acid (TEAA) with His	409.2
Total essential amino acid (TEAA) without His	386.8
% TNEAA	55.3
%TEAA with His	44.7
% TEAA without Histidine	42.3
Total neutral amino acid (TNAА)	553.4
%TNAА	60.5
Total acidic amino acid (TAAA)	250.5
Total basic amino acid (TBAA)	111.6
Total sulphur amino acid (TSAA)	41.9
%TAAA	27.4
%TBAA	12.2
% TSAA	4.58
% Cys inTSAA	48.9

Table 6. Amino acid score

Amino acid	Amino acid score	Whole egg score	FAO/WHO,1973 score	Pre-School child (2-5yrs)
Isoleucine	0.99	0.39	0.55	0.79
Leucine	1.38	0.59	0.70	0.74
Lysine	0.68	0.60	0.68	0.64
Methionine + cysteine	1.20	0.94	0.76	1.04
Phenylalanine + tyrosine	1.47	2.44	1.78	1.70
Threonine	0.99	1.06	1.34	1.59
Valine	1.05	1.05	1.57	2.26

were very close to 100% but still higher than that of whole hen's egg score (060)(60%). The value of lysine (0.68) was observed to be higher than that of whole hen's egg and the same with the value recommended by FAO/WHO [77].

4. CONCLUSION

It can be concluded that the composite sample flour is nutritionally rich in some minerals and essential amino acids useful for human physiological development. The formulation of sorghum–soy composite meal flour through fortification has positively improved the nutritional potential, nutrient density, protein solubility and digestibility of the sorghum-soy meal flour.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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