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

1. Abstract

The proximate, minerals, functional properties, in-vitro multi enzyme protein digestibility of fortified sorghum with soybean composite meal flour have been determined using standard analytical methods. The sample contained moisture (3.52%), ash (3.40%), crude fat (27.44%), crude protein (39.33%), crude fibre (5.66%) and carbohydrate (22.65%). 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 protein. The average total amino acid in the composite sample was 915.5 mg/g crude protein and the total average percent essential amino acid was 44.7% (with histidine), 42.3% (without histidine). The composite sample would provide the required amino acids for the pre-school children (2-5yrs) since the values obtained were higher than 100% required standards recommended.

Keywords: Chemical, In-vitro Multienzyme Digestibility, Amino acid, Fortified, Sorghum, Soybean, composite

2. Introduction

Plant resources are used predominantly by the developed and underutilized 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 and of the correct nutritional quality to cater for daily needs of the citizens (1). The nutritional status of the population plays a key role in the human resource development of a country. Human resource development is caused by nutrition improvement (2). In Nigeria, plant sources are the cheapest and the reliable way of protein intake. 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 of 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 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, 6, 7, 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 380mm-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 whilst a temperature of 28^0C 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 and most importantly as raw material for brewing liquor (9, 10) and value – added food products (11). It is also widely grown in dry-land agricultural systems in arid and semiarid specific zones of northern and northeastern parts of China. In those areas, the excessive consumption of water andover application of inorganic fertilizer 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 disease and cardiovascular disease (14,15).

Soybean (*Glycine max*) is an annual herbaceous species that typically grown to roughly 1 meter in height and may be branched (16) is one of the most prevalently grown and used oilseeds. It uses range 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 contents among legumes family and is often converted into various food products by fermentation with the aid of mould on bacteria to improve their flavor as well as nutritive value. In Nigeria, soy bean can be locally and industrially extracted and formulated as “soy-ogi”, it is a protein formulated as a weaning food for infants and 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.. 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

3. Materials and Methods

Sample collection and preparation

Sorghum (reddish brown) and soybean (white) grains were purchased from the main market in Akure, Ondo State in 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 composite fortified sample was then packaged in a sample glass bottle and stored in a freezer prior to analyses.

Determination of proximate composition

The moisture was determined using air-oven at temperature of 106^{o}C for 1 hour while the ash content was analyzed using a muffle furnace at 550^{o}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 500cm^3 conical flask; 200 cm^3 of boiling 1.25% H_2SO_4 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 cm^3 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^{o}C and then allowed to cool in the desiccator and weighed; later placed in the muffle furnace at 300^{o}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.

CHO = 100- [% Moisture+ % Fat +%Ash + % Crude Fibre + % Crude Protein] -------- (1).

Determination of mineral composition

1.2 grammes of the sample mixture was weighed into crucible and placed in a muffle furnace at a temperature of 550^{0}C for 6 hours to obtain a complete ash. The ash was dissolved in aqua regia and made up to mark in a 100 cm^3 volumetric 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) using phosphovanado molybdate method according to AOAC (20).

Determination of Functional Properties

The water and oil absorption capacities of the samples were determined using the method of Beuchat (21). 10 cm^3 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 the supernatant left after centrifuging was noted. Water bound was calculated from the difference in the initial volume of the solvent used and the 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 method of Lin et. al. (22), Sathe and Salunkhe (23). A 2.0g sample flour was weighed with 100 cm^3 distilled water and blended for 30 seconds using Kenwood food mixer at high speed. After complete dispersion, vegetable oil of density 0.880g per cm^3 was added to 5 cm^3 portions 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^{o}C and the value obtained was 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.

The slight modified procedure of Sathe et. al. (24) was used to determine the least

gelation concentration. Sample slurries range of 2 – 20% w/v was prepared in 5 cm^3 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^oC. The least gelation concentration was determined as the concentration which did not slip when the test tubes were heated.

The method of Coffman and Garcia (25) was employed to determine foaming capacity and stability. 1g of the sample was whipped with 50 cm^3 distilled water for 5 minutes in a Kenwood blender and later poured into a 100 cm^3 graduated flask to measure the foaming capacity/stability.

Determination of protein solubility

The protein solubility was determined using the method described by ige et al. (26). Composite flour (0.2 g) thoroughly mixed with 10 cm^3 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 and12 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 (27). The percentage nitrogen was converted to crude protein by multiplying 5.7.

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 cm^3 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 37^0C. 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 cm^3 of the multi enzyme solution was then added to the suspension with constant stirring at 37^0C. 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. (28) as follows: $Y = 210.46 – 18.10 x$ ----------(2) .

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

Determination of Amino acid

The amino acid profile was determined using the method described by Spackman et. al. (29). The sample was dried to constant weight and then defatted using Soxhlet extractor. After the defatting process, the defatted sample (2g) was weighed in a glass ampoule; 7 cm^3 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 present at 105±5^oC 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^oC rotary evaporator. The residue was dissolved in 5mL of acetate buffer (pH 2.0) and stored in specimen bottles which were kept in the freezer. The hydrolysate (7.5µL) was dispensed into the cartridge of the Technicon Sequential Multi-Analyser (TSM) using a syringe. The TSM analyser is designed to separate and analyse neutral, acidic and basic amino acids of hydrolysate. The amount of amino acids was obtained from the chromatogram peaks. The whole analysis lasted foe 76 hours and the gas flow rate was 0.50 cm^3 per minute at 60^oC with reproducibility consistent within ±3%.
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. 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):

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

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

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

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

$$\text{Concentration (g/100g crude protein)} = NH \times NH/2 \times S_{std.} \times C \text{ ------- (5)}$$

$$\text{where } c = \left\{ \frac{\text{Dilution X 16}}{\text{Sample wt. (g) X } N_2\% \text{ X 10 X Vol. loaded}} \right\} \div NH \times W \text{ (N-leu)-----(6)}$$

Where

NH = Net Weight
W = Width
N-leu = Norleucine

Estimation of Quality of Dietary protein

The quality of dietary protein can be in various ways (30), but basically, it is the ratio of available amino acid in the food or diet compared with needs expressed as ratio (31, 32, 33). The equation 7 was used to calculate the essential amino acid score:

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

4. Results and Discussion

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 fortified sorghum with soy bean has a low moisture content (3.52%), this is an added advantage as the quality is enhanced for a long time because the low moisture content prevents microbial spoilage and pest attack during storage. The moisture content is in close agreement with those obtained for gourd seeds (3.46%) (34), for melon seeds (5.0%) (35), fluted pumpkin (5.0%) (36) but relatively lower than those of water melon (37) and soybean (4.12%) (38). The average value of ash content of the sorghum fortified with soybean sample is 3.64%. The value for the total ash showed that it was low and may therefore preclude a reasonably high quality of minerals in the composite sample.

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	22.65

The fat content was 27.44% which was lower than 30.8% reported by (39) for *Brachystegia eurycoma* seed and also higher than values reported for *Cactus per cladades* (2.30%) (40), pigeon pea (6.49 – 6.57%) (41) and (6.96%) *Caesalpinia pulcherrima* (42) respectively.The crude fat value of sorghum fortified with soybean composite flour (27.44%) obtained in this study was far higher than that. obtained for sorghum in the range (2.10-7.60%) (43). This shows that there was an improvement/enhancement over the unfortified sample of sorghum. The crude fat content does not qualify unfortified sorghum as oil rich crop when compared with groundnuts (44) but fortification makes it richer in oil. Food legumes in general have higher concentration of fat than cereals (44). 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 to 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, 45, 46). The crude protein (39.33%) was found to be comparably higher than some other leguminous seed flour such as *Brachystegia curycoma* (35.8%) (39), *Sphenostylis sternocarpa* (18.55%) (47), African yam bean varieties (48), 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%) (Khalil eta la.,1984). Crude fibre also known as roughage consists mainly cellulose, hemicellulose a heterogenous group in which pentosan usually predominate over lignin, pectic and cutin substances (Salunkhe et al; 1985). The average crude fibre for fortified sorghum with soy bean composite sample was 5.66%. The carbohydrate of human diet are primarily derived from plant materials 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 22.65% which appears that fortified sorghum with soy bean 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%) (Balarabe et al., 2017).

Mineral composition of sorghum fortified with soy bean composite sample

The values for mineral composition of sorghum fortified with soybean composite 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 system 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 (Ogungbenle, 2003) and some enzymes. Zinc is another essential mineral which has a concentration of 2.34 mg/kg. The mineral content of Manganese (0.08 mg/kg), Copper (0.04 mg/kg), 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 value of 0.53 obtained for [K/(Ca+Mg)] milliequivalent was far less than 2.2. It has been recommended that the value of [K/(Ca+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.

Functional composition 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 showed 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 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 formation. 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 retention ability of the products (59). The OAC of composite sample was 114.24%. This present value of OAC was higher than those of water leaf (32.4%) (37), benniseed (45.5%) (60), cowpea (46.0%), soy flour (84.4%) and wheat flour (84.2%) (22), sorghum (55.0%) (61) and pigeon pea flour (45.70%) (62) but higher than those of *Celosia spicata* (63), dried Roselle flower calyx (496%) reported by Oluwagbenle (64) and 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 stabililty (%)	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%, cowpea (11.5%) (60), quinoa (9.0%) (1), soy flour (16.0%, 14.6%) and pigeon pea (68%, 20%) (62), *Habiscus sabdariffa* calyx (16%) as reported by Oluwagbenle (64) 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 (47.69%) of the composite sample was higher than those of *Habiscus sabdariffa* calyx (5.0%) (64) , benniseed (30.0%) (1) which shows that it is useful for binder formulation and colloidal foods. This Indicates that the composite flour may be good substitute for some legumes as food additive. The emulsion stability was 50.00% implies that the composite sample has high emulsion stability, it implies that it is a good 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%) as reported by Ogungbenle (1). 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 (66). The low gelation concentration observed may be asset in the use of these flour samples for the formulation or as additive to other gel-foaming materials in food products (67). The result of the lowest gelation of sorghum fortified with soybean (2%) shows 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/cm^3. The solubility of proteins is the manifestation of the equilibrium between protein – solvent and protein – protein interactions (68).The dependence of pH on the protein solubility of sorghum fortified with soybean flour is depicted in Figure 1. The sorghum fortified with soybean is high at both acid and alkaline media. For the composite sample, the minimum solubility was at pH 4.0 and maximum at pH 11. Figure 1 indicated the minimum protein solubility at pH 4.0 which corresponds to the isoelectric point of the protein since proteins are least soluble at their isoelectric points. The observed pH of minimum protein solubility was lower than those of pearl millet (pH 6.0) (60), and also lower than those of pigeon pea (pH 5.0) (62), fluted pumpkin seed (pH 4.0) (36) 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 the formulation of carbonated beverages (69) and low-acid foods (1).

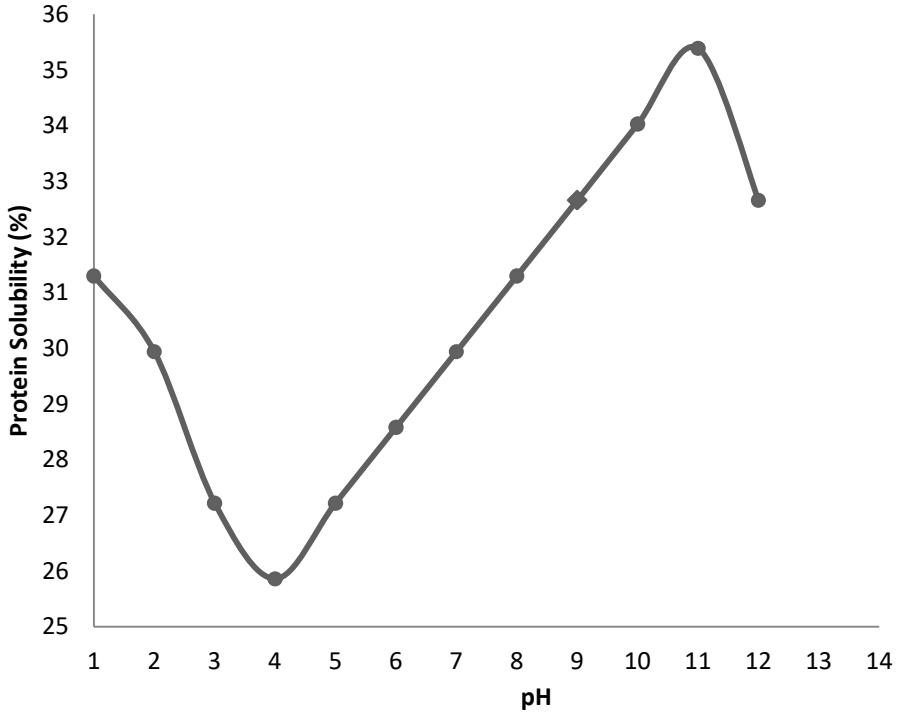

Fig. 1: Protein solubility profile of the composite sample flour.

The plot of pH on the in-vitro protein multi enzyme digestibility of sorghum fortified with soybean composite flour is depicted in Figure 2. The average digestibility value of the composite sample flour was 60%. The value of 60% was found to be lower than those values reported for dehulled African nutmeg (78.44%) (70), pigeon pea (77%) and heat –treated pigeon pea (84%) (65) and *Afzelia africana* (77.5%) (71). From Fig.2, it can be shown that

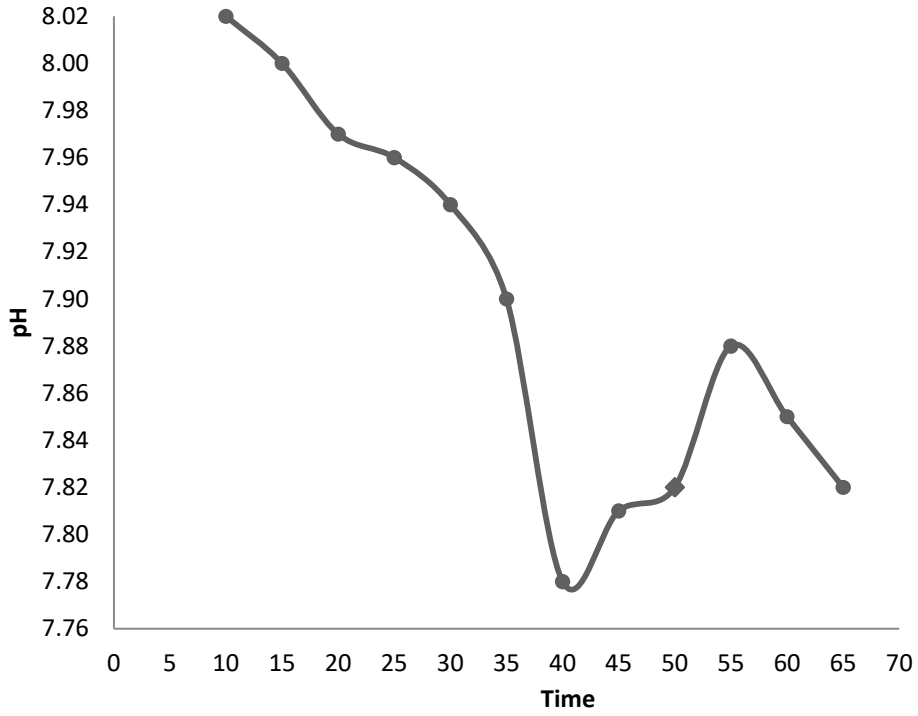

Fig. 2: In – Vitro protein digestibility of fortified sorghum with soy bean.

the pH dropped rapidly as the time increased during the hydrolysis. The protein digestibility increased as the pH decreased as obtained by Ogungbenle and Ebadan (58) 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 the least amino acid with the value of 21.4 m/g crude protein. It was observed that the present result was similar to those reported for oil seeds (72) and pearl millet fortified with soybean (64) where aspartic and glutamic acid 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. It has been observed that

Maple syrup urine disease (MSUD) is an inborn aberration of metabolism of some amino acids that results to brain damage and early death that can be avoided by diets 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 increase choline metabolism since alcoholics require more choline and may be deficient in choline which 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).

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

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. This was found within the range reported for dehulled African yam bean (AYB) (702.86 – 917.48 mg/g crude protein) (73) 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. The result of the TEAA in the composite sample studied was slightly lower than the 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) (74). The average percent TNEAA in the sample was 55.3% while the average percent TEAA was 44.7% (with histidine) and 42.3 % (without histidine). It indicated 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) (75).

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 (TNAA)	553.4
%TNAA	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

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

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

Table 6 showed that the lysine was the limiting amino acid 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 (74, 76, 77) and the present result showed that sorghum fortified with soybean is a good source of the essential 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 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 (78).

5. Conclusions

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

7. References

1**.** Ogungbenle H.N. (2003). Nutritional evaluation and functional properties of quinoa flour. *Int. J. Fd. Sci. and Nutrition,* 54, 153 – 158.

2. Zammurud S, Abid H and Muhammad A (2009). Determinats of food acceptance and micro-nutrients deficiency in pre-schoolars: A case study of households from Karachi, Pakistan. *Pak. J. Nutri*., 8(4) 321-326

3. Ihekoronye A I and Ngoddy PO (1985). Integrated Food Science and Technology for the tropics. Macmillan Publisher Ltd., London.

4. Eka, O. U. (1978). Chemical evaluation of nutritive value of soy paps and porridges, the Nigerian weaning foods. *Food Chem.* 3, 199-206

5. Gbego, J. (1987). Essais d'amelioration de la valeur nutritive de l'akassa beninois. These d' akassa beninois. These d' Ingenieur Agronome, 90pp. FSA/UNB, Abomey-Calavi: Benin.

6. Akobundu, E. N. T. and Hoskins (1987). Potential of corn-cowpea mixture as infant foods. *J. Food Agric*. 1, 111-114.

7. Cheryan, M., McCune T. D. Nelson, A. I., Ferrier, L. K. (1979). Preparation and properties of soy-fortified cereal weaning foods. *Cereal Chem.* 56 (6), 548-551.

8. Egounmlety, M., Aworh, O. C., Akingbala, J. O., Houben, J. H. and Nago, M. C. (2002). Nutritional and sensory evaluation of tempe-fortified maize –based weaning foods. *Int. J. Fd. Sci. and Nutrition,* 53, 15 – 27.

9. Liu, C., Gong X, Zhao G, Soe Htet MN, Jia Z, Yan Z, (2021). Liquor flavor is associated with the physicochemical property and microbial diversity of fermented grains in waxy and non-waxy sorghum (*Sorghum bicolor*) during fermentation. *Front. Microbiol*. 12:618458. doi:10.3389/fmicb.2021.618458

10. Maw Ni Soe Htet, Baili Feng, Honglu Wang, Lixin Tian and Vivek Yadav (2022). Comparative assessment of nutritional and functional properties of different sorghum genotypes for ensuring nutritional security in dryland agro-ecosystem. *Frontiers in Nutrition,* 9:1048789, doi:10.3389/fnut.2022.1048789

11.Duvvada SK and Maitra S(2020). Sorghum –based intercropping system from agricultural sustainability. *Indian J. Nat. Sci.* 10:20306-13.doi :10.3390/plants11121574

12. Liang D, Lu X, Zhuang M, Shi G, Hu C, Wang S (2021). China's greenhouse gas emissions for cropping systems from 1978-2016. *Sci. Data*, 8, 171

13. Xu J, Wang W, Zhao Y (2021). Phenolic compounds in whole grain sorghum and their health benefits. *Foods,* 10:1921, doi:10.3390/foods10081921

14. Cardoso LD, Pinheiro M, Martino SS, Pinheiro-Sant'Ana HS, Pinheiro-Sant'Ana HM (2017). Sorghum (*Sorghum bicolor* L): Nutrients, bioactive compounds and potential impact on human health, *Crit.. Rev. Food Sci. Nutri.* 57, 372-390

15. Yun X, Zhang PZ, Robyn D W, Fang Z X, (2019). Sorghum grain: from genotype, nutrition and phenolic profile to its health benefits and food applications. *Compr. Rev. Food Sci. Safety.*18:2025-46,doi:10.1111/1541-4337.12506.

16. https://milnepublishes.geneseo.edu.

17. https://www.science.direct.com

18. Oluwagbenle HN (2020). Nutritional evaluation of pearl millet fortified with soybean flour. *Chem. Res. J.*, 5 (4), 143-151

19. Pearson, D. (1976). Chemical Analysis of Foods, 6th edn., Pp 6-9, London, Churchill.

20. AOAC (2012): Official Methods of Analysis, 16th edn. Washington, DC: Association of Official Analytical Chemists.

21. Beuchat LT(1977): Functional and electrophoretic characteristics of succinylated peanut flour protein. *J. Agric. Food Chem.* **25**, 258-261.

22. Linn MJY, Humbert ES & Susulski FW (1974): Certain functional properties of sunflower meal products. *J. Food Sci*. 39, 368-370.

23. Sathe SK & Salunkhe DK (1981): Functional properties of the great northern bean (*Phaseolus vulgaris L*) proteins: emulsion, foaming, viscosity, and gelation properties. *J. Food Sci*. 46, 71-76.

24. Sathe SK, Deshpande SS & Sahunkhe DK (1982): Functional properties of lupin seed protein and protein concentrates. *J. Food Sci*. 47, 491-497.

26. Ige, M. M., Ogunsuwa, A.O. and Oke, O. L. (1984). Functional properties of the proteins of some Nigerian oil seeds. *J. Agric.Food Chem.* 32, 822-825

27. Wiedieselboven TE (1946). Biuret method of protein determination. *Am. J. Clin. Pathol*., 16, 40-44

28. Hsu H. W.; et al (1977). A multi enzyme technique of estimating protein digestibility. *J. Food Science*; 42, 1269 – 1273.

29. Spackman, D. H., Stein, E. H., Moore, S. (1958). Automatic Recording Apparatus for use in the chromatography of Amino acids. *Analyt. Chem.*, 30, 1191

30. FAO/WHO (1990). Protein quality evaluation, report of Joint FAO/WHO Consutation held in Bethseda, USA, 4-8 December,1989.FAO, Rome, Italy.

31. Orr ML and Watt BK (1975). Amino acid content of foods.Economics Research Report 4, USDA, Washington DC,USA

32. FAO (1970). Amino acid content of foods and biology on proteins. FAO nutritional studies no. 24 FAO Rome

33. Bender A (1992). Meat and meat products in human nutrition in developing countries, FAO Food and Nutrition paper 53, FAO,Rome

34. Ogungbenle H.N. (2006)**.** Chemical Composition, Functional Properties and Amino acids composition of some edible seeds. *Riv. Italia Sos. Grasse.* 83 (2); 204 – 208.

35. Aisegbu JE (1982). Some biochemical evaluation of fluted pumpkin seed. *J. Sci. Food Agric.*, 40, 151-155

36. Fagbemi T. N. and Oshodi A. A. (1991): Chemical composition and functional properties of fluted pumpkin seed flour (*Telfaria occidentalis*). *Nigeria Food J*.9, 26 – 32.

37. Ogungbenle, H. N. . Akinsola, A. F., Olatunde, O. C. and Lijoka, A. O. (2018). Chemical, functional properties and amino acid composition of water leaf (*Talinum triangulare*). *Chem. Res. J.*, 3 (7) 7-16

38. Temple, V. I., Odewumi, L. and Joseph, K. (1991). Soybean and soybean-bread diets. Proceedings of the 3rd regional workshop on rural development, Jos, Pp45-50

39. Amah ,C. A. K. et al (2001). Chemical and phytochemical constituents of seeds of *Brachytegia eurycoma. J. Sustain Agric. Environ*. 3 (1) 199-202

40. Lopez-Cervantes, J. Sanchez-Machado, D. J., Campas-Baypoli, O. N. and Bueno-Solano (2011). Functional properties and proximate composition of *Cactus pear cladodes* flour. *Cienci. Technol. Aliment, Campinas*, 31 (3) 654-659

41. Oshodi AA and Adeladun, MOA (1993). Proximate composition, some valuable minerals and functional properties of three varieties of lima bean (*Phaseolus lunatus Linn*.) flour. *Int. J. Fd. Sci. and Nutrition, 43, 181-185*

42. Olaofe O, Ogunlade, I and Jibuah N (2004). Proximate, mineral composition and functional properties of pride of babados (*Caesalpinia pulcherrima*) seed flour. In: Proceedings of the 27th Int. conference of Chemical Society of Nigeria Pp. 130-135

43. Abah CR, Ishiwu, CN, Obiegbura JE and Oladejo AA (2020). Sorghum grains: Nutritional composition, functional properties and its food applications. *Euro. J. Nutri. Food Safety*, 12 (5) 101-111

44. Salunkhe DK, Kadem SS and Chavan JK (1985). CRC post harvest biotechnology of food legumes. Boca raton, FL., CRC Press.

45. Akerele, I. A. and Edwards, C. C. A. (1971). An assessment of the nutritive value of a maize soy mixture, 'Soy-Ogi' as a weaning food in Nigeria. *Br. J. Nutri*. 26 (2), 177-185)

46.Ojofeitimi, E. O., Afolabi, O. A., Fapojuno, O. O., Grisson F. E. and Oke, O. L. (1984). The use of black – eyed cowpeas – maize gruel mixture. 'Ewa – Ogi' in the treatment and prevention of infantile protein malnutrition. *Nutri. Rep. Int.* 30, 841-852.

47. Sam SM (2019). Nutrient and antinutrient constituents in seeds of *Sphenostylis sternocarpa* (Hochst. Ex A Rich) Harms. *Afr. J. Plant Sci.* 13 (4) 107-112 doi:10.5897/AJFS2019.1763

48. Adeyeye, E.I., Oshodi, A.A., Ipinmoroti, K. O. (1994). Functional properties of some varieties of African yam bean II. *Int. J. Food Sci. Nutri*. 45, 116-126

49. Oluwagbenle, H. N., and Adesina, A. J. and Aremu, O. I. (2019). Comparative assessment of the proximate, mineral composition and mineral safety index of peel, pulp and seeds of Cucumber (*Cucumis sativus*). *Open J. of Applied Sciences*, 9, 691-701. Doi/10.4236/ojaapps.2009.99056

50. Khalil JK, Sawaya WN and Al-,mohammad (1984). Chemical composition and nutritional quality of sorghum flour and bread. *Plant Foods Human Nutri*. 34, 141-150

51. Balarabe MM, Mohammed SSD and Orukotan AA (2017). Physico-chemical analysis and sensory evaluation of bread produced using different indeginous yeast isolates. *Sci. Res. J.* 7 (4), 52-60

52. NRC (National Research Council) (1989). Recommended dietary allowances, 10th edn. Washington DC, USA, National Academic Press, 302 Pp.

53. Marten GC and Anderson RN (1975). Forage, nutritive and palatability of 12 common annual weeds. *Crop Sci*., 111, 829-837

54. Adeyeye, E. I. (2017). Proximate, minerals, phytochemicals, amino acids, lipids composition and some food properties of the Sclerotium pleurotus tuber-regium (Rumph.Ex FR). *J. Bio. Innov6* (3), 399-430.

55. Nieman, DC, Butterworth, DE and Nieman CN (1992). Nutrition , Dubuque, USA, Wm C., Brown publishers, Pp510

56. Hermansson MA (1979). In: Problems in Human Nutrition, Rolls J and Porter B (eds), Academic Press, New York, USA, Pp. 407

57. Sindhu R and Khatkar BS (2016). Physicochemical and functional properties of starch and flour of tartary buckwheat (*Fagopyrum tataricum*) grains. *Cytometry* 5, 315-320, doi:10.1016/j.foodchem.2015.05.122

58. Ogungbenle H.N. and Ebadan P. (2014).Nutritional qualities and amino acid profile of velvet tamarind pulp. *Bri. Biomed. Bulletin*, 2, 006-060.

59. Karl FT (2019). The Technology of wafers and waffles and adjuncts II. Cambridge, MA: Academic press, Pp 325-411. doi:10.1016/B978-0-12-809437-2.00010-1

60. Oshodi A.A., Ogungbenle H.N. and Oladimeji M.O. (1999). The Chemical composition and nutritionally valuable minerals of benniseed, pearl millet and quinoa flours. *Int. J. Food Sci. Nutri.,* 50, 325 – 331.

61. Oshodi A. A. and V. N. Enujiugha (2000). Functional properties of conophor nut. *Food Chem.* 37, 175 – 182.

62. Oshodi A.A. and Ekperigin, M. M. (1989). Functional properties of Pigeon pea (*Cajanus cajan)* flour. *Food Chem*. 34, 187 – 191.

63. Ogungbenle H.N**.** and Otemuyiwa F.F. (2015)**.** Food Properties and Amino acid composition of *Celosia spicata* leaves. *Adv. Analyt. Chem.,* 5(1) 1- 7 DOI: 10.5923/j.aac.20150501.01.

64. Oluwagbenle HN (2022). Evaluation of nutritional qualities of dried roselle flower (Hibiscus sabdariffa L.) calyx. *Chemistry Res. Journal*, 7 (4) 52-60

65. Oshodi AA, Olaofe O, Hall GM (1993). Amino acid, fatty acid and mineral composition of pigeon pea . *Int. J. Food Sci. Nutri.* 43 (4), 187-191

66. Oshodi A. A. et al; (1997). Functional properties of some varieties of African yam bean (AYB) Flour. *Int. J. Food Sci.* 489, 243 – 250.

67. Altschul A.M. and Wilcke H. L. (1985): New protein Foods Vol. 5 Academic press, Inc; Orlando Florida.

68. Kinsella JE, Damodaran S and German B (1985). Physicochemical and functional properties of oil seed proteins with emphasis on soy proteins. In: Altschul, AM, Wilcke HL eds., New protein foods, vol. 5, New York, Academic press, 107 Pp.

69. Kinsella, J. (1979). Functional properties of protein food. *Cri. Rev. Sci. Nutri.*, 7, 219-280.

70. Ogungbenle H.N. (2011).Chemical and Fatty acid Compositions Date Palm fruit (Phoenix dactylifera L) flour. *Bang J. Sci. Ind. Res.* 46(2) 255 – 258.

71. Ogungbenle H.N. and M.Omojalile (2010). Functional and anti nutritional properties,Invitro protein digestibility and amino acid composition of *Afzelia africana Pak. J Sci.Ind.Res*.53(5)265-270.

72. Olaofe O; et al; (1994). Amino acid mineral composition and functional properties of some oil seeds. *Food Chem*., 42, 878 – 881.

73. Adeyeye, E.I. (1997). Amino acid composition of six varieties of dehulled African yam beans (*Sphenostylis stenocarpa*) flour. *Int. J. Food Sci. Nutri.*, 48**:** 345-351.

74. FAO/WHO/UNU (1985). Energy and Protein Requirements. Report of a Joint FAO/WHO/UNU Expert Consultant, WHO Technical Report Series No. 724. Geneva Switzerland.

75. Altschul A.M (1958). Processed plant protein foodstuff, Pp. 713-714, Academic press, New York, USA

76. Harper LJ (1984). Food, Nutrition and Agriculture, Pp. 221, students workshop FAO, Rome, Italy.

77. Muller H. G. and Tobin G. (1980). Nutrition and Food Processing, Wesport, CT: AVI Publishing Pp 230 – 232

78. FAO/WHO (1973). Energy and protein requirements, Technical Report Series No. 522, Geneva, FAO/WHO, Pp 1.