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Nutritional Evaluation of Pearl Millet Fortified with Soybean Flour

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Abstract The chemical composition, nutritionally valuable minerals, functional properties, sugar and amino acids of fortified pearl millet (*Pennisetum typhoides)* with soybean (*Glycine max*) were studied. The results showed that the sample contained 3.56% ash, 9.66% moisture, 25.99% crude protein, 21.09% crude fat, 3.23% crude fiber and 36.48% carbohydrate. The whole pearl millet was high in maltose and D-ribose (1.44 and 1.13mg sugar in 5ml sample) but found to reduce when the pearl millet was fortified with soybean flour with the values of D- ribose (1.13 mg sugar in 5ml sample) and maltose (1.44 mg sugar in 5ml sample). The fortified pearl millet also had low contents of glucose and fructose which ranged between 0.64 and 0.69mg sugar in 5ml sample. The highest mineral was magnesium with the value of 47.33mg per 100g sample while the sample was low in copper, manganese and lead. The protein solubility was found to have minimum solubility at pH3 and maximum at pH 8. The fortified sample also contained 148.1% water absorption capacity, 117.7% oil absorption capacity, 15.50% foaming capacity, 50.53% emulsion capacity and 4.00% w/v least gelation concentration. The total essential amino acids amounted to 398.4 mg/g crude protein.

Keywords Nutritional, evaluation, pearl millet, fortified, soybean

1. Introduction

Plant sources of protein are the major ways of protein intake in many developing nations [1]. Plant resources are used predominantly by underdeveloped and developing countries to circumvent hunger and food insecurity. The world demand for plant protein sources is growing upwardly because of increasing population and improve standard of living by human race.

Soybean (*Glycine max*) was derived from Glycine soja. The geographical and historical evidences indicate that soybean was domesticated in the western part of northern china about 3000BP [2]. Changes that occurred during domestication include: increased plant and seed size, modification of a twining to erect habit and reduced dehiscence of pods. Soybean can be used for varieties of things ranging from food for human consumption to medicinal uses. Dry soybean contains 30-50% protein and it can be ground into highly nutritious flour which is rich in calcium, iron and vitamin B as well as fatty acids. The 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 invalid. Soy protein can also be spun into filaments which are made to resemble minced beef, chicken etc. by adding flavour and colour. The use of such artificial meat is on increase in the developed countries where meat is very expensive [3]. Most of the soybean grown in tropical Africa is for oil production and protein rich seed cake. Soybean prevents many diseases like cancer and menopausal problems, because it contains an estrogen hormone booster that increase libido and also prevent certain disorders. This is the reason why many scientists referred soybean to as an exceptional legume [4].

Pearl millet (*Pennisetum typhoides*) is probably of Africa in origin and mainly grown and eaten in northern zones of West Africa [5]. It is one of the major cereal crops of the semiarid regions of Africa and Asia, because of its drought tolerance and hardness, it is certainly the mainstay for millions of people of Sahel [6]. The main aim of this work is to evaluate the nutritional properties of both pearl millet and the fortified pearl millet with soybean and also compare the nutritional qualities of same.

Materials and Methods

Pearl millet (*Pennisetum typhoides*) and soybean (*Glycine max*) were purchased from the central market in Ado-Ekiti, Ekiti State in South west Nigeria. The seeds were thoroughly screened to remove the bad ones and the remaining good ones were dry-milled into flours using Marlex food blender, packaged in a polythene bag and stored in a freezer prior to further analyses.

Preparation of the fortified product

The fortified product was prepared by mixing thoroughly 50g of Pearl millet (*Pennisetum typhoides*) flour with 50 g of soybean (*Glycine max*) flour (equal proportions) using quartering method to obtain the representative whole sample.

Determination of Proximate composition

The moisture was determined using air-oven at temperature of 105^{O}C for 1 hr while the ash content was analyzed using a muffle furnace at 550^{O}C for 6 hours [7]. The sample was analyzed for crude fat and crude protein according to the methods described by AOAC [8]. The crude fiber was determined by adding 2g of the sample into 500mL conical flask; 200mL of boiling 1.25% H_2SO_4 was added and boiled for 30minutes. The mixture was filtered through muslin cloth and rinsed with hot distilled water. The sample was scrapped back into the flask and 200ml 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 and allowed to drain and dry. The residue was scrapped into a crucible, dried in the oven at 105^{O}C, allowed to cool in a desicator and weighed; then placed in muffle furnace at 300^{O}C for 30 minutes and finally allowed to cool at room temperature and re-weighed [7]. The carbohydrate content was calculated by method of difference.

Determination of Functional properties

The water and oil absorption capacities of the sample were determined using the method of Beuchat [9]. 10mL 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 oil with water in above process.

Emulsion was prepared according to method of Lin *et al* [10], Salunkhe and Kadam [11]. A 2.0g sample flour was weighed with 100mL distilled water and blended for 30 sec. using Kenwood food mixer at a high speed. After complete dispersion, vegetable oil of density 0.880g per mL was added in 5mL 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* [12] was used to determine the least gelation concentration. Sample slurries of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 % w/v were prepared in 5mL 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 4ºC. The least gelation concentration was determined as concentration which did not slip when the test tubes were inverted.

The method of Coffman and Garcia [13] was employed to determine foaming capacity and stability. 1g of the sample was whipped with 50ml distilled water for 5 minutes in a Kenwood blender and later poured into a 100ml graduated flask to study the foaming stability.

Determination of Sugar

The sugars were determined by the method of Shaffer-Somogyi sugar-thiosulfate equivalent which was described [8]. 2.5g of the sample flour was dissolved in 20ml distilled water and hydrolysed in the presence of 20ml 0.l M H_2S0_4. 5ml of the resulting solution was pipetted into 25 x 200mm test tube and then 5ml of Shaffer-Somogyi carbonate 50 reagent was added and thoroughly swirled. The test tube was placed in boiling water bath and heated for required minutes, while the test tube was removed carefully and put under a cooled water bath and allowed to cool for 4 minutes. The cap on the test tube was removed and 2ml KI-$K_2C_20_4$ were also added gently into the test tube.The mixture was mixed thoroughly to ensure that Cu_20 is dissolved and allowed to stand in cold water bath for 5 minutes with mixing done twice during the period. The remaining mixture was later titrated with 0.005M $Na_2S_20_3$ using starch indicator. The blank was equally run as described above and then the test solution titre value subtracted from that of blank. The titration was repeated until two concordant results were obtained. The amount of sugar was calculated according to Shaffer-Somogyi's equation. For glucose, the heating time was 15 minutes;

$Y = 0.1099x + 0.048$

Where Y = mg sugar in 5ml sample and x = Titre value (ml) of 0.005M $Na_2S_2O_3$

Determination of Amino acid

The amino acid profile was determined using the method described by Spackman *et al* [14]. 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; 7ml of 6MHCl was added and oxygen was expelled by passing nitrogen into the glass ampoule sealed with bunsen burner flame and placed in an oven present at 105±5ºC 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 filterate was then evaporated to dryness at 40ºC under vacuum in a rotavapor. 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 acid was obtained from the chromatogram peaks. The whole analysis lasted for 76 minutes and the gas flow rate was 0.50mL per minute at 60ºC with reproducibility consistent within ±3%.

Results and Discussion

Table 1 shows the proximate composition of both pearl millet and fortified pearl millet with soybean flour.

Data on the proximate composition of the fortified pear millet with soybean in Table 1, indicates that the fortified product contained crude protein content (cp) (25.99%) which was improved and found to be higher than the crude protein content of pearl millet flour (11.4 ± 0.9%) [15], quinoa flour (13.50%) [16] and the three parts of cucumber (22.3%) reported by Oluwagbenle *et al* [17]. This shows that after fortification, the quantity of protein increased almost more than twice.

The fat content of pearl millet (7.6%) was increased when soybean was fortified with pearl millet. This value was found to be higher than the fat content of pearl millet without soybean (7.6 $\pm$ 0.2%) [15], quinoa flour (6.3 $\pm$ 0.5%) (16) and *Bidens pilosa* leaves (7.49%) [18] but lower than those of the oil seeds; *Terracarpidium conophorum* (50.6%). C*ucumeropis edulis* (43.7%), *Citrulus vulgaris* variety 1 (47.7%) [19] and *Adenopus breviflorus* seed flour [20]. This value for the fortified pearl millet currently reported was higher than water leaf (7.45%) [21].

Table 1: Proximate composition of pearl millet and fortified pearl millet with soybean flour [15]

Proximate	*Pearl Millet	Fortified pearl millet + soybean
Moisture	10.2 ± 0.6	9.66
Protein	11.4 ± 0.9	25.99
Fat	7.6± 0.2	21.09
Ash	1.8 ± 0.3	3.56
Fibre	3.1 ± 0.5	3.23
CHO	56.9 ± 0.6	36.47

The crude fibre content of fortified pearl millet with soybean (3.23%) was higher compared to pearl millet (3.1 ± 0.5%) [15] and of Bambara groundnut flour (0.83± 0.03%) [22], but lower than *Piper guineese* (12.60%) [23], pigeon pea (3.8%) [24] and benniseed [7.9%] reported by Oshodi *et al* [15]. It has been discovered that dietary fibre has a number of beneficial effects related to its indigestibility in the small intestine [25]. This value was lower than that of cucumber seeds (4.77%) [17].

Table 2 presents the mineral contents of the pearl millet and fortified pearl millet with soybean in mg per 100g of sample. Magnesium was the highest mineral in mixed soybean and pearl millet (47.33mg per 100g sample) comparable with quinoa (23.20 ± 0.5mg per 100g sample) [15]. Potassium was found to be the next highest mineral component in fortified pearl millet with soybean (41.96mg per 100g sample). This was in close agreement with the observation of [26] that potassium was the most predominant mineral in Nigerian agricultural products. Fortified pearl millet with soybean is good for feed supplements. The values of Na (18.2mg per 100g sample), K (51.5mg per 100g sample) and Cu (0.33mg per 100g sample) in pearl millet flour were reduced substantially when fortified with soybean to corresponding values of Na (17.33mg per 100g sample), K (41.96mg per 100g sample) and Cu (0.04mg per 100g sample) respectively. While Ca (4.90mg per 100g sample), Mg (10.5mg per 100g sample) and Zn (0.45mg per 100g sample) in pearl millet were increased to Ca (11.88mg per 100g sample), Mg (47.33mg per 100g sample) and Zn (3.79mg per 100g sample), these minerals are the essential minerals needed for body. This indicated that the fortification had improved the qualities of the raw pearl millet flour.

Table 2: Mineral composition of pearl millet and fortified pearl millet with soybean flour (mg/100g) [15]

Minerals	*Pearl millet	Fortified pearl millet + soybean flour
Sodium (Na)	18.2 ± 0.2	17.33
Potassium (K)	51.5 ± 2.0	41.96
Calcium (Ca)	4.90 ± 0.8	11.88
Magnesium (Mg)	10.5 ± 0.6	47.33
Iron (Fe)	0.55 ± 0.2	0.58
Manganese (Mg)	0.0 8 ± 0.03	0.04
Copper (Cu)	0.33 ± 0.3	0.04
Zinc (Zn)	0.453 ± 0.7	3.79

Table 3 shows the functional properties of fortified soybean and pearl millet. The water absorption capacity (148.13%) was in close agreement with that of quinoa (147%) [16], but higher than those of soybean flour (130%) [10], pearl millet (115%) [15], water leaf (137.5%) [21] and lower than that of benniseed (182%) [15]. The high water absorptivity reported in the present study suggested that soybean fortified pearl millet may be used in the formulation and fortification of some foods such as sausage, doughs, processed cheese, soups, baked products etc [24, 27].

The oil absorption capacity for fortified pearl millet with soybean (117.7%) was higher than the values obtained for pigeon pea flour (89.70%) [24], three varieties of lima bean flours (82.30 - 91.50%) [28], wheat flour and soybean flour (84.20 and 84.40%) [10] and *Piper guineese* (106.75%) [23]. Oil absorption capacity is important since oil acts as flavor retainer and increases the mouth feel of foods [29].

Table 3: Functional properties of pearl millet and fortified pearl millet with soybean flour [15]

Functional Properties (%)	*Pearl millet	Fortified pearl millet + soybean flour
Water Absorption Capacity	115.0 ± 0.05	148.1
Oil Absorption Capacity	54.5 ± 0.20	117.7
Emulsion Capacity	89.0 ± 0.50	50.53
Emulsion Stability	34.0 ± 0.40	50.00
Foaming Capacity	11.30 ± 0.01	15.5
Foaming Stability	1.50 ± 0.20	3.00
Least gelation concentration (w/v)	12.0	4.0

The foaming capacity for fortified pearl millet with soybean (15%) was higher than dehulled full-fat *Adenopus breviflorus* seed flour (8.03%) [20]. It was lower than those of soybean flour (66%), sunflower flour (600%) [10] and pigeon pea (68%) [24].

Table 3 also shows the emulsion capacity and stability of the fortified pearl millet with soybean (50.53 and 50%). These values were low compared with the values of benniseed (63.0%), pearl millet (89.0%) and quinoa (104.0%) [15], but higher than those of wheat flour (7.00 - 11.00%) and soybean flour (18.00%) [10]. This indicates that the flour may be useful as additive for the stabilization of fat emulsions in the production of sausage, soup and cake [30].

The least gelation concentration of fortified pearl millet with soybean (4.00% w/v) was lower than those of lupin seed flour (14% w/v) [12] and full-fat fluted pumpkin (36% w/v) [31].

The variation of protein solubility with pH is shown in Figure 1. Protein solubility is one of the important functional properties which measure the solubility of the protein in solution or suspension. The graph indicates minimum protein solubility at pH 3 for fortified pearl millet with soybean and maximum protein solubility at pH 8 respectively while that of the whole pearl millet had only minimum protein solubility at pH 6 but without maximum protein solubility [15].

It has been observed that the solubility of dietary proteins in any food substance provides a good index for the potential or limitation of the protein; as a functional ingredient especially in food processing industries [32]. The minimum (pH = 3) and maximum (pH = 8) protein solubility of the fortified pearl millet with soybean would make it useful in the formulation of both acidic and mild alkaline food products in the food industry.

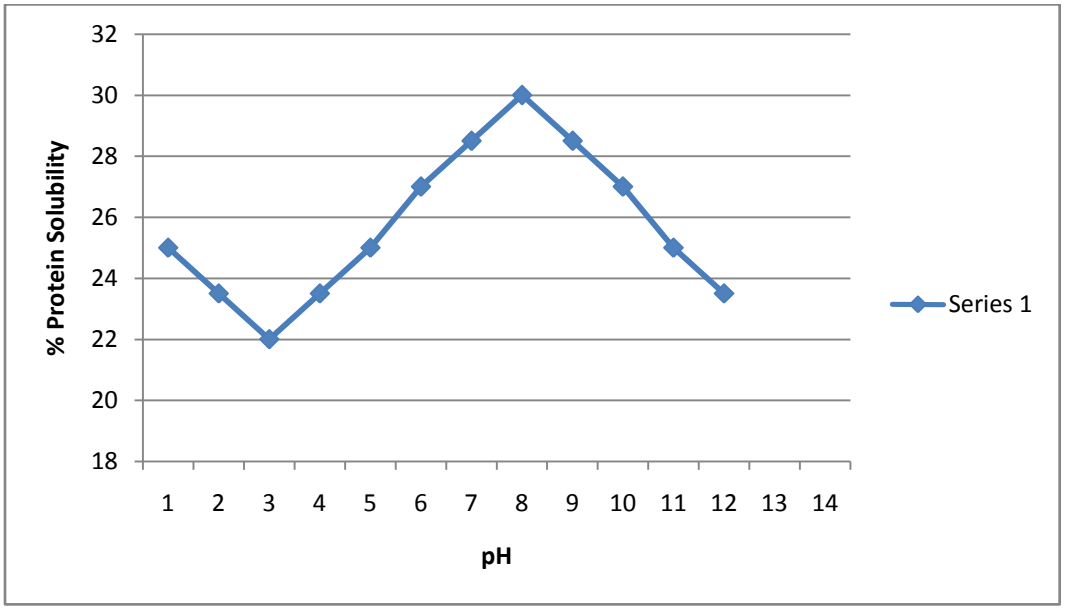

Figure 1: Protein solubility profile of fortified pearl millet with soybean

Table 4 presents the individual sugars in the fortified pearl millet with soybean in mg sugar in 5ml sample. Maltose is a disaccharide and found to be the most predominant sugar in the mixed soybean and pearl millet (1.44mg sugar

in 5ml sample). The value for fructose in fortified pearl millet with soybean was 0.69mg sugar in 5ml sample. It was observed that the values for all sugars determined were reduced after fortification of the pearl millet with soybean. The values of maltose and fructose of the fortified pearl millet were lower than that of V*elvet tamarind* pulp; maltose (1.72mg sugar in 5ml sample) and fructose (1.04mg sugar in 5ml sample) [33]. These sugars are nutritionally essential and are constituents of cell nuclei. This suggests that diets containing fortified pearl millet with soybean may probably be recommended for maturity onset diabetes patients due to their low amount of fructose and glucose.

Table 4: Sugar contents (mg sugar in 5ml sample) of pearl millet and fortified pearl millet with soybean flour [15]

Sugar	*Pearl millet	Fortified pearl millet + soybean flour
Glucose	0.70 ± 0.10	0.64
Fructose	0.74 ± 0.10	0.69
D-ribose	1.28 ± 0.06	1.13
D-galactose	0.93 ± 0.07	0.86
Maltose	1.63 ± 0.09	1.44

Tables 5 and 6 showed the results of the amino acids composition (mg/g) of raw pearl millet [6] and the fortified pearl millet with soybean flour. Glutamic acid was the predominant amino acid in both pearl millet (22.1mg/g) [6] and fortified pearl millet with soybean (156.0mg/g) presently reported (Table 5). The quality of protein in pearl millet was improved after the fortification process. The value of glutamic acid in the fortified pearl millet was found to be higher than that of water leaf (57.87mg/g) [21]. The value of histidine in pearl millet (2.8mg/g) [6] was increased as the pearl millet was fortified with soybean (31.5mg/g). The value of histidine in the fortified pearl millet was within the range of 18-36 suggested pattern of histidine [34]. It is worth noting that histidine is essentially needed by the children for their normal growth and physiological development. Children may have poor growth rate if histidine is absent in their diets [35].

Table 5: Amino acid composition of fortified pearl millet with soybean flour (mg/g) [6, 38]

Amino acids	*Pearl millet	Fortified pearl millet + soybean flour	Suggested pattern of essential amino acid requirements for infants range[a]
Lysine	2.8	65.0	53-76
Histidine	2.4	31.5	18-36
Arginine	3.9	67.3	
Aspartic acid	8.7	112.5	
Threonine	4.2	32.6	40-50
Serine	5.3	28.0	
Glutamic acid	22.1	156.0	
Proline	6.8	35.9	
Glycine	3.2	29.7	
Alanine	8.8	37.4	
Cystine	1.2	13.2	29-60
Valine	6.0	36.9	44-77
Methionine	2.3	12.6	29-60
Isoleucine	4.4	36.0	41-53
Leucine	11.5	78.8	83-107
Tyrosine	2.4	31.7	58-118
Phenylalanine	5.6	37.7	58-118

The results on Table 6 also indicated that the total essential amino acid in fortified pearl millet amounted to 398.4mg per g which was lower than those of soybean 444.00mg per g [36] and pigeon pea; 436.10mg per g [37] but found higher than that of raw pearl millet (102.3mg/g) [6]. In addition, the value of 398.4mg per g was on the low side of the range (408-588mg per g) of the total essential amino acids requirement for infants [38].

Table 6: Percentages of the essential, acidic, basic and neutral amino acids of fortified pearl millet with soybean flour (mg/g crude protein)

Amino acids	Crude protein (mg/g)
Total Amino acids	842.8
Total non-essential amino acids (TNEE)	444.4
Total Essential Amino acids (TNEAA) with Histidine	398.4
Total Essential Amino acids without Histidine	366.9
Total Acidic amino acids (TAAA)	268.5
Total Basic amino acids (TBAA)	163.8
Total Neutral amino acids (TNAA)	410.5
Percentage of total non-essential amino acids (%TNEAA)	52.73
Percentage of total essential amino acids (%TEAA) with Histidine	47.27
Percentage of total essential amino acids without Histidine	43.53
Percentage of total acidic amino acids (%TAAA)	31.86
Percentage of total basic amino acids (%TBAA)	19.44
Percentage of total neutral amino acids (%TNAA)	48.71
Total Amino acids	842.8
Total non-essential amino acids (TNEE)	444.4
Total Essential Amino acids (TNEAA) with Histidine	398.4
Total Essential Amino acids without Histidine	366.9
Total Acidic amino acids (TAAA)	268.5
Total Basic amino acids (TBAA)	163.8
Total Neutral amino acids (TNAA)	410.5
Percentage of total non-essential amino acids (%TNEAA)	52.73
Percentage of total essential amino acids (%TEAA) with Histidine	47.27
Percentage of total essential amino acids without Histidine	43.53
Percentage of total acidic amino acids (%TAAA)	31.86
Percentage of total basic amino acids (%TBAA)	19.44
Percentage of total neutral amino acids (%TNAA)	48.71

Conclusion

It can be concluded that the fortified pearl millet with soybean can be recommended for baby food supplements and as weaning food for infants because of the presence of good nutrients, essential minerals and amino acids that soybean added to the raw pearl millet through the fortification process.

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