International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 3; Issue 6; November 2018; Page No. 176-184

Ingredient optimization by response surface methodology and nutritional evaluation of bread from wheat, millet and soya meal flour blends

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Abstract

This study investigated some nutritional properties of flour blends from wheat, whole millet and soya cake. Optimization of flour blends was done using Central Composite Design (CCD) of response surface methodology (RSM) (Design Expert version 6.0.8) to determine proportions of soya cake and whole millet flours, using soya cake flour (10–20%) and whole millet flour (5–10%) as variables. Proportion of wheat flour (%) in each blend was obtained by difference. Blends generated from the design were evaluated for protein and crude fibre and three blends with highest protein and fibre contents were selected for further studies. Samples were evaluated for phytate and mineral contents, mineral-mineral and phytate-mineral molar ratios, amino acid profiles and predicted protein quality indices. Bread was also produced from the flour samples and evaluated for protein quality and consumer acceptability. Protein and fibre contents of blends increased as percentages of soya cake and whole millet flours increased. Sample WMS$_3$ {(70.00(wheat flour):20.00(soya cake flour):10.00(whole millet flour)} had highest values for phytate (42.33mg/100g) and all phytate-mineral molar ratios (6.09 for zinc, 0.018 for calcium and 0.783 for iron), but values for these parameters were within acceptable limits for all flours samples. Sample WMS$_1$ {(70.43(wheat flour):22.07(soya cake flour):7.50(whole millet flour)} had highest total amino acid for both flour (93.15g/100g of protein) and bread (95.24g/g of protein) samples respectively, as well as for most protein quality indices expect protein efficiency ratio (3.68 for flour sample). Bread sample WMS$_3$ (70.00(wheat flour):20.00(soya cake):10.00(whole millet flour) had highest consumer acceptability mean scores for all sensory parameters even though all three samples received acceptable scores for all parameters.

Keywords: bread, flour blends, ingredient optimization, nutritional evaluation, response surface

1. Introduction

Bread is a fermented baked confectionery product made predominantly from white wheat flour with the addition of water, yeast and salt and other optional ingredients such as fat, milk, egg, etc. (Adebato-Oyetoro *et al*., 2016) [1]. It is an indispensable staple food consumed by all categories of people all over the world including Nigeria, providing dietary energy and a host of other nutrients including proteins, vitamins Bs and E as well as minerals (Dewenttinck *et al*., 2008). Bread is the second most popular staple food in Nigeria after rice, even though the country does not produce wheat from which flour for break-making is milled (Shittu *et al*., 2007) [44]. The increasing popularity of bread as a major staple in different parts of the world, especially in Africa is due partly to increasing population and urbanization and partly as a result of its convenience (Olaoye *et al*., 2006) [35].

However, due to dwindling resources in many countries, especially in Africa, it is becoming increasingly difficult to source necessary foreign exchange with which to pay for imported wheat, the dominant ingredient for bread-making. In addition, when wheat flour is hydrated, it produces a visco-elastic structure called gluten which is responsible for the production of consistent dough and vesiculated crumb texture of bread (Onyango *et al*., 2015, He and Hosney, 1991) [39]. However, gluten has been implicated in the high incidence of celiac disease, and for those with this disease maintain a gluten-free diet is vital for good health (Hyslop, 2018) [19]. Moreover, wheat milling for the production of while flour, often results in the loss of most of the nutrients contained in the germ, hull and bran of the grain such as vitamins, minerals and fibre (Akinjayeju, 2015) [4].

Substitution of wheat flour, with flours from indigenous non-wheat plant food commodities such as other cereal grains and legumes in bread making, will alleviate some of the problems associated with the use of 100% wheat flour. Moreover, incorporation of flours from locally-available plant food commodities in bread formulations will boost agriculture and create jobs for the teeming populations, especially in Africa. Legumes have become an excellent addition to wheat in bread-making in view of their high nutritional values, especially in terms of dietary proteins, minerals, vitamin Bs and lysine and tryptophan, the two limiting essential amino acids in cereal grains, including wheat (Okoye and Okaka, 2009, Ndife *et al*., 2011) [33, 29]. Legumes can therefore complement cereals when they are blended at optimum ratio. Bread with good physico-chemical and nutrition properties, as well as excellent consumer acceptability had been produced by adding legumes, roots and other cereals to wheat flour as earlier reported (Okoye and Okaka, 2009, Onoja *et al*., 2011, Igbabul *et al*., 2014) [33, 29, 20].

The importance of protein and fibre in improving nutritional status has been highly acknowledged. High protein and fiber diets are associated with fewer digestive disorders, reduced rate of colon cancer, better blood-sugar control, and lower blood-cholesterol levels (Montonen *et al*., 2003, Lairon *et al*., 2005) [27, 24]. The aim of the research is to evaluate some nutritional properties of flour blends from wheat, soya meal and whole millet flours using response surface methodology. The study also evaluated the nutritional and sensory properties of bread produced from the flour blends.

2. Materials and Methods

2.1 Materials and sources

The materials used in the study were wheat flour, defatted soya meal and whole millet. Wheat flour and whole millet were purchased from Oyingbo Retail Market on Lagos Mainland, while defatted soya meal was obtained from Agro Allied Nig. Ltd., Ibadan, Nigeria.

2.2 Preparation of samples

Defatted soya meal was cleaned to remove dirt, milled in a fabricated-grinding machine and sieved using a Test Sieve Shaker. Wheat flour was also sieved and the materials that passed through sieve of mesh size 212µm were collected and packaged in high-density polythene and stored at room temperature for further studies. Whole millet flour was produced using a modified method of Olaoye *et al*., 2015) [34]. Blends of flours were produced using the Central Composite Design (CCD) of response surface methodology (RSM) (Design Expert version 6.0.8) to determine the proportions of defatted soya and whole millet respectively, using defatted soya flour (10–20%) and whole millet flour (5–10%). The proportion of wheat flour (%) in each blend was obtained by difference, that is, {100 – (% defatted soya + % whole millet)}.

2.3 Determination of protein and fibre contents of flour blends

The protein and fibre contents of the flour blends generated by optimization were determined using standard AOAC (2006) [6] method and the three blends with the highest protein and fibre contents were selected and used for further studies.

2.4 Phytate and mineral contents

Phytate content of each sample was determined using anion-exchange method as described by Ma *et al*., 2005), while mineral contents for Ca, Zn and Fe were measured by atomic absorption spectrophotometer (AAS) (Analytikjena AG, Germany) according to the method of Hernandez *et al*. (2004) [17].

2.5 Phytate-mineral molar ratios

The mole of phytate-mineral molar ratio for each sample for calcium, iron and zinc was determined by the method described by Norhaizan and Nor Faizadatul (2009) [31], which involved dividing the amount of phytate and each mineral with their respective atomic weight (phytate: 660g/mol; Fe: 56g/mol; Zn: 65g/mol; Ca: 40 g/mol). The molar ratio between phytate and mineral was then obtained by dividing the mole of phytate with the mole of minerals.

2.6 Amino acid profiles of flour and bread samples

Extraction and analysis of amino acid profiles of the flour and bread samples were carried out using the modified methods described by AOAC (2006) [6] and Danka *et al*. (2012) [9] respectively.

2.7 Predicted protein quality indices of flour and bread samples

The amino acid profile for each flour blend was used to calculate the total Amino Acid compositions of each flour blend. These include:

Total amino acid = sum of all amino acid present in each sample (TAA)

Total essential amino acids = sum of all essential amino acids in each sample (TEAA)

Total non-essential amino acids = sum of all non-essential amino acids (TNEAA)

Ratio of total essential amino acids to total amino acids (TEAA/TAA)

Ratio of total essential amino acids to total non-essential amino acids (TEAA/TNEAA)

Essential Amino Acid Index (EAAI) of each flour sample was calculated by using the ratio of test protein to the reference protein for each eight essential amino acids plus Histidine in the equation below, as quoted by Ijarotimi and Keshinro (2013) [22].

$$\mathrm{EAAI} = 10\sqrt{\frac{100a \times 100b \times 100c \times 100d \times 100e \times 100f \times 100g \times 100h \times 100i \times 100j)}{(av \times bv \times cv \times dv \times ev \times fv \times gv \times hv \times iv \times jv)}}$$

Where (a,b,…….j) a represents Lysine, Threonine, Valine, Methionine, Isoleucine, Phenylalanine, Histidine, Tryptophan, Leucine and (Methionine + Cystine) in test sample and av, bv,……..jv, represent content of the amino acid in standard protein% respectively.

Nutritional index of each flour and bread sample calculated by the method quoted by Ijarotimi and Keshinro (2013) [22], using the equation below.

$$\text{Nutritional Index (\%)} = \frac{\text{EAAI x \% Protein}}{100}$$

Where; EAAI is the Essential Amino Acid Index of each sample

Protein efficiency ratio of each flour and bread sample was estimated using the regression equation quoted by Ijarotimi and Keshinro (2013) [22] as given below:

$$\mathrm{PER} = -0.468 + 0.454(\mathrm{LEU}) - 0.105(\mathrm{TYR})$$

Biological value of each flour and bread sample was calculated according to regression equation cited by Mune *et al*. (2011) [28], using the equation below:

$$\mathrm{BV} = 1.09(\mathrm{EAAI}) - 11.7$$

2.8 Production of bread samples

Bread samples were produced by the straight dough method using a slightly modified method and recipes of Cauvain (2015) [23]. All ingredients were added at the mixing stage and kneaded to obtain consistent dough. The dough was scaled to 200g dough pieces, the dough pieces were placed in baking pans smeared with vegetable oil and was covered for the dough to proof for 40mins at 30ºC and oven-baked at 160ºC for 20 minutes, cooled and packaged for further analysis.

2.9 Sensory evaluation of bread samples

Sensory evaluation of the bread samples was carried out using an untrained laboratory panel of 20 members, who are familiar with bread and selected from students and lecturers of the Food Technology department, Yaba College of Technology. A 9-point hedonic scoring test was used ranging from 1 for disliked extremely to 9 for liked extremely to evaluate for aroma, taste, crust color, crumb texture and overall acceptability as described by Akinjayeju (2017) [5].

2.10 Statistical analysis

The responses of the panelists from sensory evaluation were converted to numerical values and analyzed statistically (SPSS) by the analysis of variance (ANOVA). Means of triplicate determinations are reported and separated using Duncan's Multiple Range Test SPSS at $p < 0.05$ significant level.

3. Results and Discussion

3.1 Protein and fibre contents of flour blends

The protein and crude fibre contents of the 13 blends generated by response surface methodology are shown in Table 1. From these results, protein and fibre contents of blends increased as proportions of soya flour and whole millet flours increased and proportion of wheat flour reduced. This could be attributed to the relatively high content of protein in soya flour and high fibre contents of both soya cake and whole millet flours compared to wheat flour (Saleh *et al*., 2013) [41]. Igbabul *et al*. (2014) [20] also reported progressive increase in the protein content of bread from composite flours from wheat, soybeans and banana as soybean flour proportion increased in the composite flours. Similar observations were also made by Nwaigbo (2013) [32], who reported increases in the protein contents of weaning foods formulated from some indigenous cereals and legume blends, and in bread from whole wheat-soya flour blends Ndife *et al*. (2011) [29]. The three blends with the highest protein contents in this study are those containing the highest proportion of soya flour compared to other blends.

Increases in the protein contents of wheat-cassava composite biscuit enriched with soy flour as the proportion of soy flour in the composite flours increased have also been reported by Oluwamukomi *et al*. (2011) [36]. The relatively high content of fibre in millet compared to other cereals has been previously reported by Saleh *et al*. (2013) [41] and Fasasi (2009) [14], which is responsible for the increase in the fibre contents of the blends with higher proportions of whole millet flour. The relatively high content of RUN 2 despite its low content of whole millet flour is most probably due to the high content of fibre in soybean flour which is 20% in this blend. Similar result was obtained by Igbabul *et al*. (2014) [20], who reported that protein and fibre contents of bread from composite of wheat, defatted soya and banana flours increased with increased substitution of defatted soya flour. As indicated in Table 1, the three blends with the highest protein and fibre contents are RUNS 2(75.00:20.00:5.00) with protein and fibre contents of 20.45% and 3.98%), RUN 4(70.43:22.07:7.50) with protein and fibre contents of 21.15% and 3.75% and RUN 8(70.00:20.00:10.00) with protein and fibre contents of 20.40% and 4.24% respectively, which were used for further studies.

3.2 Phytate-mineral ratio

The phytate-mineral molar ratios for calcium iron and zinc are shown in Table 2. Phytate is one of the common anti-nutritional factors in plant foods. In cereal grains like maize, wheat and millet, phytate is present mostly in the germ and bran which are removed during milling unlike in legume grains in which phytates are associated with proteins, and can be regarded as stores for phosphate and mineral nutrients that are important for plant nutrition and especially vulnerable during germination. While some anti-nutritional factors may have some benefits (Bora, 2014) [7], most of them however produce harmful effect in humans and animals by impairing intake, ingestion and utilization of other foods nutrients especially minerals. Phytates are known to complex zinc, iron, magnesium and calcium ions in the digestive tract, and can cause mineral ions deficiency in animals and humans (Rosalind *et al*., 2010) [40]. As shown in Table 3, There is significant difference ($p < 0.05$) in the phytate content of the flour samples with sample WMS_3 (70.00(A): 20.00 (B):10.00(C)) having the highest phytate content while sample WMS_2 {75.00(A):20.00(B):5.00(C)}had the least phytate value.

The relatively low phytate value of this sample is mostly due to its lower percentage of whole millet flour compared to other two samples. As proportions of whole millet and soya flours increased in the blends, the phytate contents also increased. This is due to high amounts of phytate in whole millet and soya bean flours when compared to wheat flour as previously reported by Fasasi (2009) [14] and Bora (2014) [7]. There is significant difference ($p < 0.05$) in the phytate-mineral ratios among the samples for all three minerals with sample WMS_3 (70.00(A):20.00(B):10.00(C)) having the highest values due to the high contents of phytate in this sample. Phytate-calcium molar for the flour samples ranged from 0.012 for sample WMS_2 to 0.018 for sample WMS_3, phytate-iron molar ratio ranged from 0.466 for sample WMS_2 to 0.783 for sample WMS_3, while phytate-zinc ratio was between 4.19 for sample WMS_2 to 6.09 for sample WMS_3. In general, sample WMS_2 showed the lowest phytate-mineral molar ratios for the three minerals, while sample WMS_3 had the highest values. This is most probably due to its relatively higher proportions of whole millet and defatted soya flours. All the samples have phytate-mineral ratios which are within the standard of phytate:iron (< 1), phytate:zinc (< 18), and phytate:calcium (< 0.17) Rosalind *et al*. (2010) [40] and Hurrell (2004) [18]. This means that there will be no problem of bio-availability of zinc, calcium and iron in these samples.

3.3 Amino acid profiles

The amino acid profiles, of the flour samples for both essential and non-essential amino acids are shown in Table 3. All the flour samples contain the 20 characteristic amino acids in varying proportions. A major determinant of protein quality in foods is the content, quality and availability of its essential amino acids since they play important role for growth, reproduction and maintenance in the human body. With respect to essential amino acids, the leucine had the highest value of 7.07g/100g of protein for sample WMS_2 {75.00(A):20.00(B):5.00(C)}, while sample WMS_3 (70.00(A):20.00(B):10.00(C)) has the least leucine value of 6.43g/100g protein. Leucine is considered, most probably, the only dietary amino acid that can stimulate muscle protein synthesis Etzel (2004), and has important therapeutic role in stress conditions like burn, trauma and sepsis FAO/WHO/UNU (2007). On the other hand, the least essential amino acid for all samples are tryptophan for which sample WMS_2 {75.00(A):20.00(B):5.00(C)}had the least value of 1.03g/100 protein and 1.19g/100g protein for sample WMS_1 (70.43(A):22.07(B):7.50(C).

Methionine is also low in the flour samples, especially sample WMS_2 (1.56g/100g protein) and sample WMS_1 (1.73mg/100g protein). From the amounts of tryptophan and methionine these amino acids in the samples, they could be regarded as two limiting amino acids. This however may not be the case if the chemical scores of these amino acids are used. These results are in agreement with the observation of Scott *et al*. (2004) [42], that the limiting amino acid in legumes are tryptophan and methionine, in view of the high contents of soya bean flours in the samples, as well as the observations of Jiddere and Filli (2015) [23], that methionine is the third limiting amino acid in normal maize after lysine and tryptophan and it is the first limiting amino acid in legumes. This has also been corroborated by the study of Omwamba and Mahungu (2014) [37], that methionine as well as lysine, are generally known to be limiting amino acids in cereals and legumes respectively.

The total essential amino acid (TEAA) in the samples ranged from 30.53g/100g protein for sample WMS_3 (70.00 (A): 20.00(B):10.00(C) to 32.05g/100g protein for sample WMS_1 (70.43(A):22.07(B):7.50(C). The relatively high total essential amino acid in this sample is most probably due to the relatively high proportion of defatted soya bean flour compared to other two samples. As observed by Nicole *et al*. (2010) [30], addition of soya protein source to cereal flours resulted in increases in the protein content, enhanced amino acid balance in the extruded products of the cereal-soya flour blends and consequently improved the nutritional value of the products.

With respect to non-essential amino acids, all nine essential amino acids are present in the flour samples with glutamate having the highest values in all samples when compared with other amino acids, with values 26.34g/100g protein, 26.76g/100g protein and 24.91g/100g protein for the flour samples respectively (Table 3). This amino acid plays an important role in amino acid metabolism because of its role transamination reactions and it is necessary for the synthesis of key molecules such as glutathione which are required for removal of highly toxic peroxides and poly glutamate folate cofactor (Scott *et al*., 2010 and Omwamba and Mahungu, 2014) [37]. The next high non-essential amino acid in the flour samples is proline with values 9.3mg/100gm protein, 8.02g/100g protein and 8.73g/100g protein for samples in the three samples respectively. Proline helps in proper formation of collagen and cartilage in the body, and also in offsetting the effect of ageing in the human body. Total non-essential amino acid (TNEAA) followed similar trend as for the TEAA, with values 93.15g/100g protein, 91.37g/100g protein and 90.48g/100g protein for samples WMS_1, WMS_2 and sample WMS_3. This is mostly due to the relatively high proportion of soya flour in sample WMS_1 compared to other two samples. Similar results were reported in a previous study by Young (2011) [46].

The amino acid profiles of bread samples are shown in Table 5, which followed similar trends as for the flour samples (Table 3). Results showed that like flour samples, bread samples also had highest leucine contents compared to other essential amino acid. For this amino acid, the values for flour and bread samples are 6.53mg/100gm protein and 6.44mg/100gm protein, 7.07g/100g protein and 7.08g/100g protein and 6.43mg/100gm protein and 6.44g/100g protein for samples WSM_1, WSM_2 and WSM_3 respectively. Samples WSM_2 and WSM_3 had the same leucine contents for flour and bread samples, while sample WMS_1 for bread had slightly higher leucine content (6.70g/100g protein) when compared to the value for flour sample (6.53g/100g protein). This means that baking temperature used did not produce any adverse effect on this essential amino acid.

This trend was also observed for most other essential amino acids, because methionine and tryptophan which are the limiting amino acids in the flour samples are also deficient in the bread samples, while the values for phenylalanine, valine and isoleucine also ranked next to leucine in both flour and bread samples respectively. Total essential amino acid (TEAA) is almost the same in both flour and bread samples for sample WSM_1 (32.05g/100g protein and 32.03g/100g protein), whereas there were marginal increases in the TEAA for bread samples compared to flour samples for samples WSM_2 (32.03g/100g, 31.81g/100g protein), and WSM_3 (30.97g/100g protein, 30.53g/100g) respectively. These results are in agreement with the observations of Jiddere and Filli (2015) [23] and Omwamba and Mahungu (2014) [37], that extrusion cooking produced minimal reduction and even in some cases marginal increases in most amino acids, especially threonine, arginine, valine and phenylalanine. These results show that bread samples produced for the flour samples will provide substantial percentages of the daily essential amino acids requirements for the consumers.

Non-essential amino acids also showed similar trends as for essential amino acids for both flour and bread samples. While glutamate had highest values in flour samples, it is also the largest non-essential amino acid in bread samples and had slightly higher values for bread samples compared to flour samples, except for sample WSM_1 for which glutamate values for flour and bread samples are almost the same (26.34g/100g protein and 26.80g/100g protein). Proline and aspartate are other two non-essential amino acids with high values in both flour and bread samples, both amino acids having almost equal values for flour and bread samples. Aspartate, glutamate and glycine are precursors of nucleic acids, which are parts of DNA. Consequently, the presence of these amino acids in the samples will be beneficial to the consumer. Total non-essential amino acid (TNEAA) also showed similar trends as for the individual amino acids for both flour and bread samples, with marginal increases in TNEAA for bread samples compared to flour samples (Table 6).

3.4 Predicted Protein Quality Indices

The predicted protein quality indices of the flour samples are shown in Table 4, while values for bread samples are presented in Table 6. For flour samples, there were significant differences ($p < 0.05$) among the samples for most protein quality indices except for ratios TEAA/TNEAA, TNEAA/TAA and TEAA/TAA for which there was no statistical significance ($p < 0.05$). Sample WMS_1 had the highest values for almost all predicted protein quality indices except for PER for which this sample had the least value. The relatively higher protein quality indices in sample WMS_1 could be due to its relatively higher proportion of soya flour compared to other two samples. Omwamba and Mahungu (2014) [37] and Nicole *et al*. (2010) [30] reported in previous studies that addition of soy protein to cereal flour increased protein content and improved amino acid balance of extruded products. This results in the complementation of cereal-legume combinations which will provide high quality protein comparable to or higher than reference proteins as quoted by Ijarotimi and Keshinro (2013) [22].

Aromatic amino acids for the flour samples ranged from 6.58g/100g protein for sample WSM_2 to 7.84g/100g protein, Sulphur amino acid values are 3.46g/100g protein for sample WSM_2 to 4.39g/100g protein for sample WSM_3, the values for ratio TEAA + Hist + Arg/TAA are not significantly different ($p < 0.05$) with values 37.31%, 37.94% and 36.31% for samples WSM_1, WSM_2 and WSM_3 respectively. The total aromatic amino acid values obtained in this study are within the range of 6.50g/100g protein and 7.64g/100g protein reported for Bambara bean flour and protein concentrate respectively but much higher for total sulphur amino acid values of 1.10g/100g protein and 0.67g/100g protein for the same products (Mune *et al*., 2011) [28].

The percentage ratio TEAA/TAA in the flour samples are 34.11%, 34.81% and 34.12% for samples WSM_1, WSM_2 and WSM_3 respectively, which are far higher than the ideal for children (26%) and adults (11%), but slightly lower than 39% considered adequate for infants FAO/WHO/UNU (2007) [33]. The values for this protein quality index obtained in this study are however a little lower than the values for some animal protein sources (Adeyeye, 2005 and (Adeyeye and Adamu, 2005) [2, 3]. The EAAI values for the flour samples ranged between 70.78% for sample WSM_2 to 72.10% for samples WSM_1, predicted biological values ranged from 65.41 for sample WSM_2 to 66.89 for sample WSM_1, while nutrition index values were between 14.47 for sample WSM_1 and 15.25 for sample WSM_3. These results show that for these protein quality indices, sample WSM_1 had the highest values while sample WSM_2 had the lowest value. This is most probably as a result of the relatively higher proportion of soya flour in this sample compared to other two samples as earlier observed by Young (2011) [46] and Friedman and Brandon (2001) [15].

The EAAI, predicted PER, biological value and nutrition index obtained in this study are higher that values obtained for these protein quality parameters for bitter cola flours by Ijarotimi *et al*. (2015) [21] and fermented and germinated popcorn, but lower than values for raw popcorn reported by Ijarotimi and Keshinro (2013) [22]. In terms of nutritional quality, a protein-based food is considered to be of good when it has a protein efficiency ratio of 2.7, biological values >70% and essential amino acid index (EAAI) >0.70. The flour samples used in this study meet these standards except for biological value for which the flour samples had slightly lower values. As indicated in Table 5, the limiting essential amino acids in the flour samples are tryptophan and methionine.

With respect to bread samples (Table 6), the predicted protein quality indices followed almost similar trends as for flour samples, with significant differences ($p < 0.05$) among samples for most parameters. Sample WSM_1 had the highest values for most indices except for ratio TEAA/TNEAA TEA/TAA and predicted PER. Also, similar to amino acid profiles, bread samples did not show any depletion in the values of protein quality indices, but rather marginal increases in bread samples compared to flour samples. For instance, bread sample WSM_1 had 72.36%, 66.375 and 67.17 for EAAI, ratio TNEAA/TAA and biological value, while the corresponding values for its flour sample counterpart are 72.10%, 65.59% and 66.89. This indicates that high baking temperatures had no adverse effect on these parameters. However, the value for predicted PER for this sample for bread reduced drastically from 3.68 for its flour sample to 2.82 for bread sample, a trend which was also observed for other two samples. This means that baking temperature had no appreciable adverse effect on PER.

3.5 Sensory Evaluation

The mean scores of the sensory parameters of the bread samples are shown in Table 7. While sample WSM_1 is significantly different ($p < 0.05$) from two other samples for most parameters measured, the other two samples were not significantly different from each other. All bread samples had high mean scores (> 6) for most sensory parameters except sample WSM_2. The high mean scores for most parameters obtained in this study deviate from some results of Udofia *et al*. (2013) [45], who reported that bread with lower proportion of soya bean flour of about 10% had high mean scores for sensory parameters. The mean scores for crumb texture may have been affected by the proximate compositions and amount of each component of the flour samples. Serrem *et al*. (2011) [43] had reported that crumb texture of bread is affected by some factors which include fibre, protein (as gluten), starch, degree of dough development. The relatively high men score for crumb texture of bread sample WSM_1 compared to other two samples is therefore due to the slightly higher percentage of soya bean flour and fibre content of the flour sample from which this bread sample was produced. This result agrees with the observations of Eiman *et al*. (2008), that increased fibre content resulted in hard crumb texture of bread and Ndife *et al*. (2011) [29], who reported high crumb texture of bread with increased substitution level of soya bean flour of up to 30%. The high proportions of soya bean flour in these samples did not reflect in their scores for aroma, especially for sample WSM_1 {70. 43(wheat): 22.07(Soya flour): 7.50(whole millet)}, which had high acceptability scores despite its relatively higher percentage of soya flour compared to other two samples. Food legumes have been associated with beany flavour (Okoye and Okaka, 2009) [33]. Baking temperature may have inactivated the enzyme responsible for the breakdown of linoleic and linolenic acids thereby preventing the production of hydroperoxides responsible for beany flavour (Serrem *et al*., 2011) [43]. The higher mean score in crust colour for sample WSM$_1$ {70.43(wheat):22.07(Soya flour):7.50(whole millet)} could be due to its slightly higher fibre and protein contents from soya bean flour, both of which had been observed to contribute to browning during bread making (Mohsen *et al*., 2009) [26].

Table 1: Protein and fibre contents of wheat, whole millet and soya meal flour blends at different levels generated by response surface methodology (%)

Runs	A	B	C	Protein	Crude fibre
	77.50	15.00	7.50	18.68	2.63
2	75.00	20.00	5.00	20.45	3.98
3	77.50	15.00	7.50	18.45	2.65
4	70.43	22.07	7.50	21.15	3.75
5	77.50	15.00	7.50	18.58	2.61
6	80.00	10.00	10.00	16.90	2.58
7	77.50	15.00	7.50	18.64	2.59
8	70.00	20.00	10.00	20.40	4.24
9	73.96	15.00	11.04	18.64	2.74
10	81.04	15.00	3.96	18.71	2.52
11	77.50	15.00	7.50	18.56	2.59
12	85.00	10.00	5.00	16.95	2.42
13	84.57	7.93	7.50	16.20	2.45

A = Wheat flour; B = Defatted Soya flour; C = Whole millet flour

Table 2: Phytate and phytate-mineral molar ratios of wheat, whole millet and soya meal flour blends

Parameters/Samples	WMS$_1$	WMS$_2$	WMS$_3$
Phytate (mg/100g)	34.00±0.273^{b}	24.17±0.45^{c}	42.33±0.75^{a}
Phytate-Calcium	0.015±0.001^{b}	0.012±0.001^{c}	0.018±0.001^{a}
Phytate-Iron	0.593±0.03^{b}	0.466±0.03^{c}	0.783±0.04$_{a}$
Phytate-Zinc	5.24±0.33^{b}	4.19±0.25^{c}	6.09±0.32^{a}

Means of triplicate determinations reported.
Means with similar superscripts along rows are not significant ($p > 0.05$)

WMS$_1$ = 70.43(A):22.07(B):7.50(C)
WMS$_2$ = 75.00(A):20.00(B):5.00(C)
WMS$_3$ = 70.00(A):20.00(B):10.00(C)
A = Wheat Flour; B = Soya meal flour; C = Whole millet

Table 3: Amino acid profiles of wheat, whole millet and soya meal flour blends (g/100g Protein)

Parameters/Samples	WMS$_1$	WMS$_2$	WMS$_3$
Essential amino acids			
Histidine	2.70 ± 0.26^{b}	2.86 ± 0.45^{a}	2.32 ± 0.12^{c}
Isoleucine	4.88 ± 0.15^{a}	4.82 ± 0.68^{a}	4.38 ± 0.20^{b}
Leucine	6.53 ± 0.25^{b}	7.07^{a} ± 0.75	6.43^{b} ± 0.15
Lysine	2.81 ± 0.15^{a}	2.77 ± 0.05^{a}	2.75 ± 0.24^{a}
Methionine	1.73 ± 0.20^{b}	1.56 ± 0.88^{c}	2.11 ± 0.20^{a}
Phenylalanine	4.89 ± 0.15^{a}	3.74 ± 0.18^{b}	4.37 ± 0.18^{c}
Threonine	2.43 ± 0.17^{c}	3.32 ± 0.25^{a}	3.02 ± 0.08^{b}
Tryptophan	1.19 ± 0.16^{a}	1.03 ± 0.15^{b}	1.06 ± 0.05^{b}
Valine	4.89 ± 0.15^{a}	4.64 ± 0.45^{b}	4.09 ± 0.14^{c}
TEAA	32.05 ± 0.12^{a}	31.81 ± 0.35^{b}	30.53 ± 0.28^{c}
Nonessential amino acids			
Alanine	3.44 ± 0.15^{a}	3.46 ± 0.20^{a}	3.40 ± 0.15^{a}
Arginine	3.76 ± 0.24^{b}	3.45 ± 0.35^{c}	4.22 ± 0.36^{a}
Aspartate	5.14 ± 0.36^{b}	5.09 ± 28^{c}	5.27 ± 0.56^{a}
Cysteine	2.05 ± 0.16^{b}	2.08 ± 0.15^{b}	2.28 ± 0.12^{a}
Glutamate	26.34 ± 0.25^{a}	26.76 ± 0.24^{a}	24.91 ± 0.36^{b}
Glycine	3.60 ± 0.32^{b}	3.95 ± 0.18^{a}	3.94 ± 0.15^{a}
Proline	9.23 ± 0.42^{a}	8.02 ± 0.25^{c}	8.73^{b} ± 0.25
Serine	4.59 ± 0.25^{a}	3.91 ± 0.18^{b}	3.86 ± 0.42^{b}
Tyrosine	2.95 ± 0.15^{a}	2.84 ± 0.20^{b}	2.34 ± 0.24^{c}
TNEAA	61.10 ± 0.24^{a}	59.56 ± 0.40^{b}	58.95 ± 0.36^{c}
TAA	93.15 ± 0.20^{a}	91.37 ± 0.12^{b}	90.48 ± 0.24^{c}

Means of duplicate determinations reported
Means with similar superscripts along rows are not significant ($p > 0.05$)
WMS$_1$ = 70.43(A):22.07(B):7.50(C)
WMS$_2$ = 75.00(A):20.00(B):5.00(C)
WMS$_3$ = 70.00(A):20.00(B):10.00(C)
A = Wheat Flour; B = Soya meal flour; C = Whole millet

Table 4: Predicted protein quality indices of wheat, whole millet and soya meal flour blends

Samples/Parameters	WMS$_1$	WMS$_2$	WMS$_3$
TEAA (g/100gProtein)	32.05±0.17^{a}	31.81±0.31^{a}	30.53±0.32^{b}
TNEAA (g/100gProtein)	61.10±0.30^{a}	59.56±0.17^{b}	58.950.15^{c}
TAA (g/100gProtein)	93.15±0.13^{a}	91.370.15^{b}	89.48±0.12^{c}
∑Sulf AA(Meth + Cys) g/100gProtein	3.78±0.15^{b}	3.64±0.13^{c}	4.39±0.14^{a}
∑AromaticAA(Phen+Tyr) g/100gProtein	7.84±0.16^{a}	6.58±0.14^{c}	6.71±0.14^{b}
TEAA+Hist+Arg/TAA (%)	37.31±0.25^{a}	37.94±0.18^{a}	36.31±0.32^{b}
TEAA/TNEAA	0.52±0.12^{a}	0.53±0.15^{a}	0.52±0.11^{a}
TNEAA/TAA (%)	65.59±0.12^{a}	65.19±0.14^{a}	65.88±0.15^{a}
TEAA/TAA (%)	34.41±0.20^{a}	34.81±0.24^{a}	34.12±0.18^{a}
EAAI (%)	72.10±0.51^{a}	70.78±0.12^{c}	71.77±0.10^{b}
Predicted PER	3.68±0.16^{b}	3.99±0.15^{a}	3.69±0.15^{b}
Predicted BV	66.89±0.14^{a}	65.45±0.07^{c}	65.81±0.11^{b}
Nutrition Index	15.25±0.02^{a}	14.47±0.03^{c}	14.64±0.05^{b}
Limiting AA* 1	Tryptophan	Tryptophan	Tryptophan
Limiting AA* 2	Methionine	Methionine	Methionine

Means of duplicate determinations are reported
Values with the same superscript along rows are not significant ($p > 0.05$)
*****: AA = Amino acid
WMS$_1$ = 70.43(A):22.07(B):7.50(C)
WMS$_2$ = 75.00(A):20.00(B):5.00(C)
WMS$_3$ = 70.00(A):20.00(B):10.00(C)
A = Wheat Flour; B = Soya meal flour; C = Whole millet

Table 5: Amino acid profiles of bread samples from wheat, whole millet and soya meal flour blends (g/100g protein)

Parameters/Samples	**WMS_1**	**WMS_2**	**WMS_3**
Essential Amino Acids			
Histidine	2.79±0.04^{a}	2.58±0.02^{b}	2.17±0.0^{c}
Isoleucine	4.95±0.15^{a}	4.83±0.05^{b}	4.29±0.03^{c}
Leucine	6.70±0.18^{b}	7.08±0.10^{a}	6.44±0.01^{c}
Lysine	2.89±0.10^{a}	2.80±0.04^{b}	2.78±0.02^{b}
Methionine	1.80±0.05^{b}	2.14±0.02^{a}	2.18±0.12^{a}
Phenylalanine	4.09±0.08^{c}	4.22±0.12^{b}	4.67±0.04^{a}
Threonine	2.48±0.12^{c}	3.34±0.05^{a}	3.03±0.02^{b}
Tryptophan	1.30±0.01^{a}	1.24±0.02^{b}	1.29±0.01^{a}
Valine	5.03±0.04^{a}	4.67±0.02^{b}	4.12±0.0.03^{c}
TEAA	32.03±0.15^{b}	32.90±0.18^{a}	30.97±0.15^{c}
Non-essential Amino Acid			
Alanine	3.61±0.06^{a}	3.33±0.02^{c}	3.41±0.02^{b}
Arginine	3.99±0.12^{b}	3.05±0.04^{c}	4.86±0.10^{a}
Aspartate	5.21±0.18^{a}	5.10±0.14^{b}	5.28±0.12^{a}
Cysteine	2.34±0.06^{a}	2.22±0.18^{c}	2.26±0.04^{bc}
Glutamate	26.80±0.20^{b}	28.74±0.04^{a}	28.71±0.10^{a}
Glycine	3.78±0.12^{c}	3.89±0.05^{a}	3.81±0.02^{bc}
Proline	9.48±0.04^{a}	8.00±0.12^{c}	8.59±0.14^{b}
Serine	4.84±0.02^{a}	3.89±0.15^{b}	3.84±0.01^{b}
Tyrosine	3.16±0.03^{a}	2.90±0.21^{b}	1.91±0.04^{c}
TNEAA	63.21±0.15^{a}	61.12±0.25^{c}	62.67±0.15^{b}
TAA	95.24±0.25^{a}	94.02±0.18^{b}	93.64±0.24^{c}

Means of duplicate determinations are reported

Means with similar superscripts along rows are not significant ($p > 0.05$)

WMS_1 = 70.43(A):22.07(B):7.50(C)

WMS_2 = 75.00(A):20.00(B):5.00(C)

WMS_3 = 70.00(A):20.00(B):10.00(C)

A = Wheat Flour; B = Soya meal flour; C = Whole millet

Table 6: Predicted protein quality indices of bread samples from wheat, whole millet and soya meal flour blends

Parameters/Samples	**WMS_1**	**WMS_2**	**WMS_3**
TEAA (g/100gProtein)	32.03±0.04^{b}	32.90±0.10^{a}	30.97±0.02^{c}
TNEAA (g/100gProtein)	63.21±0.02^{a}	61.12±0.15^{b}	62.67±0.16^{a}
TAA (g/100gProtein)	95.24±0.10^{a}	94.02±0.1^{b}	93.64±0.20^{c}
TEAA/TNEAA	0.51±0.02^{b}	0.54±0.01^{a}	0.49±0.02^{b}
TNEAA/TAA (%)	66.37±0.15^{a}	65.01±0.03^{b}	66.93±0.15^{a}
TEAA/TAA (%)	33.63±0.10^{b}	34.99±0.15^{a}	33.07±0.06^{c}
EAAI (%)	72.36±0.20^{a}	71.04±0.24^{b}	71.36±0.12^{b}
Predicted PER	2.82±0.04^{b}	3.05±0.02^{a}	2.81±0.02^{b}
Predicted BV	67.17±0.10^{a}	65.73±0.15^{b}	67.08±0.12^{a}
Nutrition Index	14.31±0.04^{a}	13.55±0.03^{b}	13.58±0.04^{b}
Limiting AA* 1	Tryptophan	Tryptophan	Tryptophan
Limiting AA* 2	Methionine	Methionine	Methionine

Means of duplicate determinations are reported

Means with similar superscripts along rows are not significant ($p > 0.05$)

*****: AA = Amino acid

WMS1 = 70.43(A):22.07(B):7.50(C)

WMS2 = 75.00(A):20.00(B):5.00(C)

WMS3 = 70.00(A):20.00(B):10.00(C)

A = Wheat Flour; B = Soya meal flour; C = Whole millet

Table 7: Mean scores of sensory parameters of bread samples from wheat, whole millet and soya meal flour blends

Parameters/Samples	**WMS_1**	**WMS_2**	**WMS_3**	**LSD**
Crust colour	7.70±0.92^{a}	6.30±0.82^{b}	6.75±0.75^{b}	0.001
Taste	6.75±0.97^{a}	6.00±0.78^{b}	6.40±1.01^{b}	0.000
Aroma	6.50±0.69^{a}	5.80±1.04^{b}	6.40±0.75^{b}	0.000
Crumb Texture	6.95±0.76b^{b}	6.35±0.78^{b}	7.45±0.93^{a}	0.000
Overall Acceptability	7.70±0.74^{a}	6.54±1.10^{b}	6.80±0.75^{ab}	0.058

Means with similar superscripts along rows are not significant ($p > 0.05$)

WMS_1 = 70.43(A):22.07(B):7.50(C)

WMS_2 = 75.00(A):20.00(B):5.00(C)

WMS_3 = 70.00(A):20.00(B):10.00(C)

A = Wheat Flour; B = Soya meal flour; C = Whole millet

4. Conclusion

This study has shown that high-protein bread of acceptable quality can be produced from blends of wheat, whole millet and de-fatted soya flours. It also showed that bread of acceptable sensory quality can be produced at substitution level of up to 30% of wheat flour with combination of whole millet and defatted soya flours. The bread produced form the flour blends have improved nutritional quality especially fibre, minerals and protein, thereby enhancing the nutritional status of consumers and reduce the prevalence of malnutrition and food insecurity in the society. The substitution level of up to 30% will reduce the need for imported wheat thereby helping to conserve foreign exchange used on wheat importation and consequently reduce the local price of the product. Future study will focus on the use of other protein sources as extenders of wheat flour for production of bread and in vivo determination of the protein quality indices through animal studies with a view to comparing with predicted protein quality indices.

Conflict of interest

We hereby confirm that there is no conflict of interest either between us as authors or between us and anyone whatsoever.

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