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AMINO ACID COMPOSITION AND PROTEIN QUALITY OF WHEAT FLOUR BISCUITS FORTIFIED WITH SOYBEAN AND BAMBARA GROUNDNUT FLOURS

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ABSTRACT: The amino acid composition and protein quality of soybean/wheat and Bambara groundnut/wheat composite flours and biscuits were investigated. The wheat flour (WF) was blended in each case with cooked, blanched and cooked/blanched soybean (SF) and bambara groundnut (BGF) flours in the ratios of 70:30 (WF:SF) and 70:30 (WF:BGF) respectively and used for amino acid analysis and production of short soft dough biscuits. The amino acid composition of the samples showed that lysine and the sulphur containing amino acids (methionine, cystine and trytophan), which are known to be limiting in both cereals and legumes were significantly improved (p<0.05) in the composite blends. The result also revealed that the amino acid profile of the blends were reduced by cooking than blanching and combined treatment effects of cooking/blanching. Similarly, the biscuits produced from the composite blends after amino acid analysis were dried, cooled and milled gradually into biscuit flours.

Keywords: Biscuits, fortification, amino acid profile, protein quality, soybean flour, bambara groundnut flour.

1. INTRODUCTION

In recent times, the increase in demand for wheat flour and other wheat products in non-temperate regions like Nigeria have necessitated the need for the production of flour from available and affordable indigenous food crops that would serve as substitutes or complement wheat flour in baked goods. Although wheat is an important cereal crop that is highly utilized in the preparation of a wide range of bakery products, it can only be produced in very limited quantities in Nigeria because of the climatic conditions. Annual production of wheat in 2004 was about 8,000 metric tonnes [1]. Many food crops like cocoyam, cassava, yam, sorghum, cowpea, soybean, maize and bambara groundnut etc can be used for partial replacement of imported wheat flour used for the production of biscuits, bread and other western style bakery products, which are very popular convenience foods among children and adolescents [2]. Several studies carried out by Okaka, J.C., et al; Iwe, M.O., et al; Onyekwere, O.O., and Mepba, H.D., *et al.* showed that the protein quality of baked goods such as biscuits, bread and cakes could be improved by supplementing wheat flour with adequately processed legume flours (such as cowpea, soybean, kidney bean and bambara groundnut), provided the objectionable beany flavour associated with baked goods containing high levels of non-bland legume flours are overcome. In effect, beany flavour associated with legume products can be drastically reduced by soaking legume seeds in water containing 0.1-1.0 percent sodium bicarbonate or citric acid followed by the application of heat treatment in order to destroy the lipoxygenase enzyme found naturally in them which catalyses the oxidation of their polyunsaturated fatty acids with the formation of hydroperoxides during processing [7-9].

Legumes are complementary protein sources to cereal grains and add nutritional value to cereal-based products [10-11].

Bambara groundnut, an indigenous African legume, appears to be one of the lesser known and utilized legumes yet to be exploited fully in the preparation of baked products such as biscuits, bread and other confectionery despite its high protein content. The level of production, processing, and utilization of soybean in many food preparations is today gaining importance because people are now aware of its nutrient content and utilization methods. However, the limited information on the amino acid profile and protein quality of biscuits fortified with soybean and bambara groundnut flours prompted this study. Therefore, the purpose of this study is to determine the amino acid composition of soybean/wheat and bambara groundnut/wheat composite flours and the protein quality of biscuits produced from them.

2. MATERIALS AND METHODS

Mature dried soybean (*Glycine max*) and black variety of bambara groundnut seeds (*Vigna subterranea (L) verde*) seeds used for this study were purchased from Owerri and Nsukka Main Markets, Imo and Enugu States, Nigeria. The wheat flour and other ingredients used for the preparation of biscuits were purchased from Owerri Main Market, Imo State, Nigeria.

2.1. Preparation of Cooked Full-Fat Soybean Flour

The cooked full-fat soybean flour was prepared according to the method of Iwe, M.O. During preparation, two kilograms (2kg) of soybean seeds which were free from dirt and other foreign particles such as stones, sticks and leaves were weighed, cleaned and soaked in 3.5 litres of potable water at room temperature (29±2^{0}C) for 8h. The soaked seeds were drained, rinsed and dehulled manually by rubbing in between palms. The dehulled seeds were then boiled with 2.5 litres of potable water in an electrically heated pot at 100^{0}C for 30min. The boiled seeds were drained, spread on the trays and dried in a hot air oven (Model ETA 224) at 60^{0}C for 6h. During drying, the boiled seeds were stirred at intervals of 30min to ensure uniform drying. The dried seeds were milled using a locally fabricated attrition mill and sieved to obtain flour that passed through a 500 micron mesh sieve. The cooked soybean flour produced was packaged in an airtight plastic container for blending and analysis.

2.2. Preparation of Blanched Full-Fat Soybean Flour

The blanched full-fat soybean flour was prepared according to the method of Iwe, M.O. During preparation, two kilograms (2kg) of soybean seeds which were free from dirt and other foreign particles such as stones, sticks and leaves were weighed cleaned and soaked in 3.5 litres of potable water at room temperature (29±2^{0}C) for 8h. The soaked seeds were drained, rinsed and de-hulled manually by rubbing in between palms. The de-hulled seeds were hot water blanched with 2.5 litres of potable water in an electrically heated pot at 85^{0}C for 20min. The blanched seeds were then drained, spread on the trays and dried in a hot air oven (Model ETA 224) at 60^{0}C for 6h. During drying, the blanched seeds were stirred at intervals of 30min to ensure uniform drying. The dried seeds were milled using a locally fabricated attrition mill and sieved to obtain flour that passed through a 500 micron mesh sieve. The blanched soybean flour produced was packaged in an airtight plastic container for blending and analysis.

2.3. Preparation of Cooked Bambara Groundnut Flour

The cooked bambara groundnut flour was prepared according to the method of Baryeh, E.A. During preparation, two kilograms (2kg) of black cultivar of bambara groundnut seeds which were cleaned from dirt and other foreign particles such as stones, leaves and sticks were weighed and soaked in 4 litres of potable water at room temperature (29±2^{0}C) for 18h with occasional change of soak water at intervals of 6h to prevent fermentation. The soaked seeds were drained, rinsed and boiled with 3 litres of potable water in an electrically heated pot at 100^{0}C for 30min. The boiled seeds were drained, spread on the trays and dried in a hot air oven (Model ETA 224) at 60^{0}C for 8h. During drying, the seeds were turned at intervals of 30min to ensure uniform drying. The dried seeds were dehulled by cracking them in a locally fabricated attrition mill followed by winnowing. The de-hulled seeds were milled using the attrition mill and sieved to obtain flour that passed through a 500 micron mesh sieve. The cooked bambara groundnut flour produced was packaged in an airtight plastic container for blending and analysis.

2.4. Preparation of Blanched Bambara Groundnut Flour

The blanched bambara groundnut flour was prepared according to the method of Baryeh, E.A. During preparation, two kilograms (2kg) of black cultivar of bambara groundnut seeds which were free from dirt and other foreign particles such as stones, leaves and sticks were weighed, cleaned and soaked in 4 litres of potable water at room temperature (29±2^{0}C) for 18h with occasional change of soak water at intervals of 6h to prevent fermentation. The soaked seeds were drained, rinsed and hot water blanched with 3 litres of potable water in an electrically heated pot at 85^{0}C for 20min. The blanched seeds were drained, spread on the trays and dried in a hot air oven (Model ETA 224) at 60^{0}C for 8h. During drying, the blanched seeds were turned at intervals of 30min to ensure uniform drying. The dried seeds were dehulled by cracking them in a locally fabricated attrition mill followed by winnowing. The dehulled seeds were milled using the attrition mill and sieved to obtain flour that passed through a 500 micron mesh sieve. The blanched bambara groundnut produced was packaged in an airtight plastic container for blending and analysis.

2.5. Flour Blend Formulation

Wheat flour (WF) was blended in each case with cooked, blanched and cooked/blanched soybean (SF) and bamabra groundnut (BGF) flours in a Kenwood Mixer (Model, NX 960 G, Kenwood, Britain, UK) in the ratios of 70:30 (WF:SF) and 70:30 (WF: BGF), respectively. The composite flours produced were individually packaged in sealed polyethylene bags and kept in a cool dry place until used for amino acid analysis and production of biscuits.

2.6. Amino Acid Analysis

The amino acid profile of the composite blends was determined by Ion Exchange Chromatography (IEC) according to the method of Hussain, M.A., using the Technicon Sequential Multisample (TSM) Amino Acid Analyzer (Technicon Instruments Corporation, New York). The samples were defatted, hydrolyzed, evaporated in a rotary evaporator before being injected into the Amino Acid Analyzer for the determination of their amino acid profile. The period of analysis for each sample was 72min and the net height of each peak produced by the chart recorder of the (TSM) (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of triplicate determinations. Norleucine was used as the internal standard.

2.7. Preparation of Biscuits

The short soft dough biscuits were prepared according to the creaming method of Akubor, P.I.A. The biscuits were produced with recipe containing high levels of fat and low levels of sugar. The recipe used for the production of short soft dough biscuits contained 100% flour, 60% fat, 40% sugar (sucrose), 4% beaten whole egg, 4% liquid milk, 2% salt and 20% baking powder. During biscuit making, the sugar and fat were initially creamed in a Kenwood Mixer (Model NX 960 G, Kenwood, Britain, UK) at medium speed until fluffy. The liquid milk and whipped whole egg were added and mixed for 30 min before the salt, baking powder and composite flours in each case were slowly added to the mixture. Thereafter, the mixture was mixed thoroughly until uniform smooth and hard consistent dough was obtained. The dough was thinly rolled on a flat wooden board sprinkled with the same flour with a wooden rolling pin to a uniform thickness (2mm) and cut out using a biscuit cutter to desired shapes of similar sizes. The cut out biscuit dough pieces were placed individually into greased baking trays and baked in a hot air oven at 220^{0}C for 30min. The baked biscuits were removed from the oven and allowed to cool at ambient temperature (29±2^{0}C) for 20min. After cooling, one and half kilograms (1.5kg) of the biscuits produced from each sample of the composite blend were collected and packaged individually in an airtight plastic container and used for the formulation of the test diets.

2.8. Formulation of Experimental Diets

The diets were formulated according to the method of Annan, N.T. Prior to the formulation of the diet, already baked short soft dough biscuits were further placed in each case into baking trays and dried in a hot air oven (Model ETA 224) at 60^{0}C for 40min. The dried biscuits were allowed cool to ambient conditions (29+2^{0}C) for 15min and then milled individually into biscuit powders using a locally made attrition mill. After milling, the crude protein content of the biscuit flours was determined using a modified micro-kjeldahl method of A.O.A.C before being used for the formulation of test diets. Based on the values of the crude protein content of the biscuit flours, the total amounts (in grams) for each diet was calculated. The diets were formulated to provide 10 percent of dietary protein regardless of the nitrogen sources [18]. The reference protein or control diet contained casein (H.J. Heinz Co. Ltd, England) as the sole source of protein. During the formulation, calculated quantity sugar (sucrose), cornstarch; vegetable oil, mineral premix and vitamin premix were added to both the control and the biscuit containing test diets to balance the diets.

Generally, one and half kilograms (1.5kg) of each diet was formulated and used for the experimental feeding study carried out with weanling albino rats for a period of 28 days. After the formulation, the test and control diets were packaged individually in an airtight plastic container and kept in the freezer until needed. The proportions of the ingredients used for the formulation of the diets based on their protein content and those used for the formulation of 1.5kg of each diet used for the experimental feeding study are presented in Tables 1 and 2.

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Table 1: Composition of Diets Made With Biscuits Fortified With Cooked, Blanched and Cooked/Blanched Soybean and Bambara Groundnut Flours.

Ingredients (%)	I	II	III	IV	V	VI	VII
Casein	10.53	-	-	-	-	-	-
Biscuit	-	52.94	51.33	51.12	67.29	69.06	66.93
Vegetable oil	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mix	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral mix	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sucrose	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Corn starch	75.97	33.56	35.17	35.38	19.21	17.44	19.57

I. - Casein based diet used as control or reference protein, II. - Diet formulated from biscuit made with 70% wheat flour and 30% cooked soybean flour, III.- Diet formulated from biscuit made with 70% wheat flour and 30% blanched soybean flour, IV. - Diet formulated from biscuit made with 70% wheat flour and 30% cooked/blanched soybean flour, V. - Diet formulated form biscuit made with 70% wheat flour and 30% cooked bambara groundnut flour, VI. - Diet formulated from biscuit made with 70% wheat flour and 30% blanched bambara groundnut flour, VII. - Diet formulated from biscuit made with 70% wheat flour and 30% cooked/blanched bambara groundnut flour.

Table 2: Composition of 1.5kg of the Diets Used For Experimental Feeding Study.

Ingredients (g)	I	II	III	IV	V	VI	VII
Casein	157.95	-	-	-	-	-	-
Biscuit	-	794.10	769.95	766.80	1009.35	1035.90	1003.95
Vegetable oil	52.5	52.5	52.5	52.5	52.5	52.5	52.5
Vitamin mix	45	45	45	45	45	45	45

Mineral mix	37.5	37.5	37.5	37.5	37.5	37.5	37.5
Sucrose	67.5	67.5	67.5	67.5	67.5	67.5	67.5
Corn starch	1139.55	503.4	527.55	530.7	288.15	261.60	293.55

The descriptions are the same as in Table 1 above.

2.9. Experimental Animals and Housing

Forty-nine weanling albino rats (Wistar strain) aged 24 days and weighing 22.40 – 32.60 grams were obtained from Animal Science Unit, University of Nigeria Teaching Hospital Enugu. The animals which were made up of twenty-six males and twenty-three females were divided into seven groups of seven rats each on the basis of body weight. They were housed individually in wire cages equipped with facilities for separation of urine and feaces and for collection of spilled feed. The rats were weighed prior to access the experimental diets and at weekly intervals to estimate change in body weight.

2.10. Experimental Feeding

The experimental rats were fed their respective diets and tap water *ad libitum* for a period of 28 days which included 2–day acclimatization period. During this period, the rats were fed with a commercial stock diet purchased from the same unit of the University where the rats were procured and no records of feed intake and weight were taken. After adaptation, the rats were fed 50g of their respective diets and tap water each day for a period of 28 days and other procedures followed were as described by Akaninwor, *et al*. Daily feed intakes were recorded for the 28 days for each group and the data generated were used in calculating biological values and net protein utilization during the period. Weight gain and feed intake for each group were used to estimate protein efficiency ratio (PER) and net protein ratio (NPR) [18].

2.11. Sample Collection Procedures

The urine and feaces were collected from each group every morning within the twenty-eight-day period of the study. Collection of urine and feaces started on day one after acclimatization and ended on the morning of day twenty-nine. The pooled urine for each group was preserved with 2ml of 0.1 N HCl and stored in sample bottles at 6^{0}C in a refrigerator until needed. The pooled feaces were also dried in a hot air oven at 105^{0}C for 24h and weighed before being ground into flour using laboratory mortar and pestle. The flours obtained were stored in sealed polyethylene bags and kept at room temperature (29±2^{0}C) until needed.

2.12. Carcass Analyses

Each rat was weighed after 28 days before being sacrificed. The weighed rats were euthanized with chloroform and then sacrificed. The internal organs (liver, heart and kidney) of each group were evacuated, weighed and returned to carcass. The pooled carcasses for each group were weighed and dried in a hot air oven at 105^{0}C for 48h. The dried carcasses were milled individually into flour using laboratory mortar and pestle. The flours obtained were stored in sealed polyethylene bags and kept at room temperature (29±2^{0}C) until needed.

2.13. Estimation of Biological Values, Net Protein Utilization, Protein Efficiency Ratio and Net Protein Ratio

The urine, feaces and carcasses of each group were analyzed for nitrogen content according to the method of A.O.A.C [17]. The values obtained for feacal, digested, urinary and retained or absorbed nitrogen from each group of rat were used in calculating nitrogen intake, biological values (BV) and net protein utilization (NPU). Weight gain and feed intake for each group were used to estimate protein efficiency ratio (PER) and net protein ratio (NPR) [18].

2.14. Statistical Analysis

The data generated were subjected to Analysis of variance (ANOVA) using statistical package for Social Science (SPSS version 10.1, 2003) to detect significant difference at $p \leq 0.05$. Significant means were separated using Turkey's least significant difference (LSD) test.

3. RESULTS AND DISCUSSION

The amino acid compositions of the composite blends are given in Table 3. Cooking and blanching treatments altered the amino acid composition of the soybean/wheat and bambara groundnut/wheat composite flours, increasing some and decreasing others. The amino acid contents of the blends supplemented with blanched and cooked/blanched soybean and bambara groundnut flours were significantly ($p<0.05$) higher than those supplemented with cooked legume flours. The general increases in the amino acid composition of the blends further explain the higher protein content and quality of the samples. The results of the amino acid composition are generally in agreement with the observations of recent investigators [20-21]. The variation in amino acid composition could influence the amino acid balance and subsequently, the protein quality. The present study also indicates that lysine and the sulphur containing amino acids (methionine, cysteine) and trytophan, which are known to be limiting in cereals and legumes, respectively are significantly improved in the blends. Therefore, it appears that the complementary effect of cereal / legume mixture improves both the protein quality and the levels of the limiting amino acids. In addition, the samples fortified with variously processed soybean flour had better amino acid profile and contained appreciably higher amounts of eight essential amino acids, particularly lysine, leucine and phenylalanine than those containing similar types of processed bambara groundnut flour. However, the blends generally could be used as protein and amino acid supplements in the formulation of a number of food products. Table 4 shows the protein quality of casein and biscuit containing diets fed on weanling albino rats. The effect of diets on the protein utilization of rats showed that the corrected protein efficiency ratio (PER) and net protein utilization (NPU) of Diet I (Casein based diet) was significantly higher than the values for all the test diets. Similarly, the PER and NPU of Diets VI and VII were found to be significantly ($p<0.05$) lower than their values for all the diets used for the study. In spite of the higher PER value of casein based diet (Diet I), the PER of the diet (Diet I) is relatively similar to that of the test diets. This similarity could be an indication that all the available proteins from different biscuit containing diets were adequately utilized for the growth of the rats. Onimawo, I.A., *et al* reported that the PER of protein of good to high quality is slightly above 2.0. However, the PER scores of the test diets used for the study meet this standard.

In addition, there were significant differences ($p<0.05$) between the net protein ratio (NPR) and biological value (BV) of casein based diet (Diet I) and all the test diets. The variation could be due to the unique amino acid balance of casein protein compared to plant proteins, which are deficient in some essential amino acids. The results also showed that Diets VI and VII had the lowest NPR and BV values. However, the NPU and BV values of the test diets generally were appreciably high compared to their values for casein based diet (Diet I) used as control or reference protein. Isichei, M.O., *et al* reported that protein of the highest quality has the NPU and BV of 100%. The BV and NPU values of the diets used for the study are relatively close to this value, which is an indication that all their available proteins were properly utilized for the growth and maintenance of the rats. Generally, the PER, NPU, NPR and BV scores of the biscuit based diets were generally lower than those of the casein containing diet (Diet I). The decrease could be due to the losses of amino acids as a result of non-enzymatic browning (Maillard) reactions, which occurred between the protein (amino acid) and the added sugar/inherent carbohydrate forming compounds during baking. This reaction has been incriminated in decreased protein activity in animals [18, 24-25]. The PER, NPU, NPR and BV scores reported here are on short soft dough biscuits containing 30 percent processed soybean and bambara groundnut flours. It is possible that the short soft dough biscuits containing 40 – 50 percent soybean and bambara groundnut flours may exhibit improved PER, NPU, NPR and BV beyond the values shown by the present study because there may be more available free amino acids in such products even after the classical Maillard reaction has taken place. Ideally, the PER, NPU, NPR and BV scores of the diets made with biscuits fortified with either cooked, blanched or cooked/blanched soybean flour were higher than those containing biscuits supplemented with variously processed bambara groundnut flour.

Essentially, the quality of protein food is nutritionally judged by its protein content and the number and amounts of essential amino acids it contains. The present study, however, shows that the use of cooked and blanched soybean and bambara groundnut flours in the fortification of biscuits generally improve the protein quality of the samples by increasing both their protein content and the levels of the limiting essential amino acids particularly lysine and sulphur containing amino acids (methionine, cystine and tryptophan), which are known to be deficient in cereal and legume products. From the results presented in Table 4, it could be easily said that the improved PER, NPU, NPR and BV scores exhibited by the biscuit containing diets compared to the values in the control diet (casein based diet) were as a result of increase in the levels of available free amino acids in the products occasioned by the complementary effect of cereal/legume mixture. Therefore, the efficiency with which a protein is used for growth and maintenance of the body is a measure of its quality which is determined by its amino acid composition digestion and utilization [22]. Even though the PER, NPU, NPR and BV scores of the casein based diet (Diet I) were higher than those of the biscuit containing diets, all the test diets recorded values that are relatively close to those of the casein based diet, which is an indication of their high potential in meeting the protein needs of children and adults who would depend on legume fortified biscuits as their sources of protein especially in regions where protein availability is a problem.

Table 3: Amino Acid Profile of Soybean/Wheat and Bambara Groundnut/Wheat Composite Blends
Amino acid Concentration in Composite Blends (g/100g protein)

	70WF:30 SF (Cooked)	70WF:30 SF (Blanched)	70WF:30 SF (Cooked / Blanched)	70WF:30 BGF (Cooked)	70WF:30 BGF (Blanched)	70WF:30 BGF (Cooked / Blanched)
Lysine*	4.47^{e}±0.42	4.64^{a}±0.44	4.85^{a}±0.47	4.32^{c}±0.40	4.64^{a}±0.42	4.68^{a}±0.45
Histidine	3.26^{b}±0.26	3.38^{b}±0.28	3.63^{a}±0.31	2.78^{c}±0.20	2.92^{c}±0.22	3.08^{b}±0.24
Trytophan*	3.86^{b}±0.34	3.92^{b}±0.35	4.04^{a}±0.37	3.32^{c}±0.27	3.48^{b}±0.29	3.62^{b}±0.31
Arginine	5.68^{b}±0.57	5.72^{a}±0.57	5.76^{a}±0.58	5.02^{c}±0.49	5.16^{c}±0.51	5.28^{b}±0.52
Aspartic acid	6.17^{b}±0.62	6.54^{a}±0.67	6.62^{a}±0.70	5.36^{c}±0.53	5.62^{c}±0.56	5.78^{c}±0.58
Threonine*	3.56^{c}±0.62	3.87^{b}±0.35	4.12^{a}±0.38	3.32^{c}±0.27	3.48^{c}±0.29	3.96^{e}±0.36
Serine	3.89^{d}±0.35	4.14^{c}±0.38	4.16^{c}±0.38	4.89^{b}±0.46	4.94^{b}±0.48	5.02^{a}±0.49
Glutamic acid	14.84^{b}±1.37	15.56^{a}±1.42	15.64^{a}±1.43	14.28^{c}±1.33	14.36^{c}±1.34	14.73^{b}±1.36
Proline	6.62^{a}±0.67	6.65^{a}±0.68	6.68^{a}±0.68	5.86^{c}±0.58	6.28^{d}±0.64	6.42^{d}±0.65
Glycine	4.28^{b}±0.40	4.32^{b}±0.40	4.47^{a}±0.42	4.13^{c}±0.38	4.24^{b}±0.39	4.28^{b}±0.40
Alanine	3.78^{a}±0.33	3.82^{a}±0.34	3.88^{a}±0.35	3.68^{b}±0.32	3.72^{b}±0.33	3.78^{a}±0.33
Cystine	3.02^{c}±0.23	3.13^{b}±0.25	3.22^{a}±0.26	2.66^{d}±0.18	2.74^{d}±0.19	2.83^{d}±0.20
Valine*	3.48^{a}±0.29	3.56^{a}±0.30	3.76^{a}±0.33	3.27^{b}±0.26	3.34^{b}±0.27	3.58^{a}±0.30
Methionine*	3.36^{b}±0.28	3.64^{b}±0.32	3.86^{a}±0.34	3.18^{c}±0.25	3.22^{c}±0.26	3.42^{d}±0.29
Isoleucine*	3.28^{b}±0.26	3.34^{b}±0.27	3.52^{a}±0.28	2.86^{d}±0.21	2.98^{d}±0.22	3.06^{c}±0.24
Leucine*	7.86^{b}±0.80	8.12^{a}±0.83	8.26^{a}±0.86	7.24^{c}±0.74	7.36^{c}±0.75	7.44^{c}±0.76
Tyrosine	4.16^{b}±0.38	4.24^{b}±0.39	4.32^{a}±0.40	4.06^{c}±0.37	4.12^{b}±0.38	4.22^{a}±0.39
Phenylalanine *	4.43^{c}±0.42	4.76^{a}±0.46	4.68^{a}±0.45	4.36^{c}±0.41	4.42^{c}±0.42	4.38^{c}±0.41

WF – wheat flour, SF – soybean flour, BF – bambara groundnut flour

Values are means ± standard deviations of triplicate determinations on dry weight basis.

Means with different superscripts within the same column are significantly different ($P<0.05$).

Essential amino acids are shown on asterisks.

Table 4: Protein Quality of Casein and Biscuit Containing Diets.

Diets	Corrected Protein efficiency ratio (PER)	Net protein utilization (NPU) (%)	Net protein ratio (NPR)	Biological value (BV) (%)
I	2.5^{a}±0.42	94.28^{a}±0.83	1.92^{a}±0.34	92.82^{a}±0.78
II	2.36^{b}±0.36	92.42^{a}±0.78	1.74^{b}±0.28	91.56^{b}±0.72
III	2.32^{b}±0.36	91.26^{c}±0.72	1.66^{c}±0.22	90.26^{c}±0.64
IV	2.26^{c}±0.28	90.16^{c}±0.64	1.62^{c}±0.20	88.86^{d}±0.62
V	2.24^{c}±0.28	88.86^{d}±0.62	1.48^{d}±0.18	88.74^{d}±0.62
VI	2.20^{c}±0.22	86.74^{d}±0.60	1.36^{d}±0.16	86.82^{e}±0.60

VII	$2.16^d±0.24$	$84.62^e±0.56$	$1.28^d±0.13$	$84.64^f±0.56$

Values are means ± standard deviations of determined parameters per group.

Means with different superscripts within the same column are significantly different ($p<0.05$).

I. - Casein based diet used as control or reference protein, II. - Diet formulated from biscuit made with 70% wheat flour and 30% cooked soybean flour, III. - Diet formulated from biscuit made with 70% wheat flour and 30% blanched soybean flour, IV. - Diet formulated from biscuit made with 70% wheat flour and 30% cooked/blanched soybean flour, V. - Diet formulated form biscuit made with 70% wheat flour and 30% cooked bambara groundnut flour, VI. - Diet formulated from biscuit made with 70% wheat flour and 30% blanched bambara groundnut flour, VII. - Diet formulated from biscuit made with 70% wheat flour and 30% cooked/blanched bambara groundnut flour.

4. CONCLUSIONS

The study showed that the amino acid profile of the composite flours were significantly ($p<0.05$) increased by blanching than the cooking treatment. The present study also reveals that lysine and the sulphur containing amino acids (methionine, cystine and trytophan), which are known to be deficient in cereals and legumes, respectively are greatly improved in the blends. In addition, the study also indicated that the PER, NPU, NPR and BV scores of rats fed on casein based diets used as control were relatively higher than those maintained on biscuit based test diets. Generally, the PER, NPU, NPR and BV values of the diets made with biscuits fortified with either cooked, blanched or cooked/blanched soybean flour were higher than those containing biscuits supplemented with variously processed bambara groundnut flour. However, the production of soybean and bambara groundnut fortified biscuits should be highly encouraged, as research has shown the products to be acceptable and have the potential to support both the growth and maintenance of the body in human and animal subjects.

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