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Amino Acid Composition and Nutritional Indices of Flour and Biscuit from Wheat and Bambara Groundnut Blends

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Abstract

The aim of this study was to determine the amino acid composition and nutritional indices of bambara groundnut, an underutilised legume for use both as flour and protein isolate in biscuit production. Sixteen (16) response surface methodology (RSM) runs were evaluated from combination of wheat flour (WF), bambara groundnut flour (BF) and bambara protein isolate (BPI). Based on the crude protein and functional properties these ratios were selected WF: BF (85.0:15.0%, 76.2:23.8% and 52.5:47.5%) and labeled as WBF1, WBF2, WBF3, respectively with WF: BPI (85.0:15.0%, 76.2:23.8% and 52.5:47.5%) as WBPI1, WBPI2 and WBPI3 for blends of wheat flour and bambara protein isolate respectively. The amino acid of the flour and biscuit from bambara groundnut was investigated. Data were analysed using one-way analysis of variance (ANOVA) and significance at $P<0.05$. The limiting and most abundant amino acids were methionine in wheat-bambara composite flour (32.73-90.24) and biscuit (58.64-131.36) and arginine in wheat-bambara composite flour (149.50-429.00) and biscuit (98.37-307.50). The essential amino acid index (EAAI) of wheat-bambara composite flour ranged between (57.16- 66.35) and (37.06 – 76.49) for biscuit. The Protein Efficiency Ratio (PER) of wheat-bambara composite flour and biscuit were (2.40-2.70) and (1.70 – 3.05) respectively. The Biological Value (BV) ranged between (50.60- 60.63) for wheat-bambara composite flour and (47.17–66.99) for biscuit. Hence, the essential amino acids in the composite flour and biscuit will adequately support growth and development in infants, children and adults.

Key words: Bambara groundnut; flour; biscuit; amino acid; nutritional indices

Introduction

The use of various underexploited food materials in product development is on the increase, probably due to growth in human population with resultant hike in prices of foods. Bambara groundnut (*Vigna subterrenea* L.) is an underutilized major source of vegetable protein in sub Saharan Africa (Adu-Dapaah and Sangwan, 2004). It is well adapted to harsher conditions and constitutes an important part of the local diet, culture and economy. Adu-Dapaah and Sangwan (2004) reported that the seed is regarded as a completely balanced food because it is rich in iron (4.9-48 mg/100 g), compared to the value (2.0-10.0 mg/100 g) for most food legumes, potassium (1144-1935 mg/100 g), sodium (2.9-12.0 mg/100 g), calcium (95.8-99 mg/100 g), protein (18.0-24.0%) with high lysine and methionine contents, ash (3.0-5.0%), fat (5.0-7.0%), fiber (5.0-12.0%), carbohydrate (51-70%), oil (6-12%) and energy (367-414 kal 100 mgG1). Lackroix *et al.* (2003) have shown that, the energy value of Bambara groundnut seed is greater than that of several other pulses. Bambara groundnuts can be eaten in many ways; immature pods can be boiled and consumed as snacks. However, at maturity the seeds become hard and require boiling for long periods. Recently, a trial of bambara groundnuts milk was carried out which compared its flavor and composition with those of milk prepared from cowpea, pigeon pea and soybean (Mkandawire, 2007).

Biscuits are confectionery dried to very low moisture content. According to Nwosu (2013), biscuit is defined as a small thin crispy cake made from dough. Biscuits are an important baked product in human diet and are usually eaten with tea and are also used as weaning food for infants. The ingredients are simple; which contain soft wheat flour, sugar, fat, eggs (Nwosu, 2013; Adegbanke *et al.*, 2019). Efforts have been made to enrich wheat with bambara groundnut to produce cake (Adegbanke and Akinnodi, 2019). Therefore, this study aimed to substitute wheat flour with bambara groundnut as available low cost crop for the poor in production of biscuit, and evaluate the amino acids composition of

Bambara groundnut. The study would provide better understanding regarding the effect of bambara groundnut as a nutritive supplement.

Materials and Methods

Materials: Bambara groundnut seeds used in the study were purchased in large quantity at Owena market, Osun State, Nigeria. The seeds were authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. All reagents used were of analytical grade.

Methods

Processing of bambara groundnut

Prior to defatting and isolation of protein, bambara groundnut was processed by modifying the method of Mune *et al*., (2011). First, the nuts were sorted manually to remove extraneous materials and diseased seeds. The selected nuts were then soaked for 6 h in water at room temperature, dehulled and dried in hot air oven at 50 ºC for 72 h. The dried nuts were milled with attrition mill to obtain flour and sieved with 84 µm mesh size. The flour was deffated by using Soxhlet extraction and n-hexane solvent for 8 h according to AOAC, (2012*)*. The sample was removed from the thimble, dried at 105 ºC, milled, sieved and packed in plastic container sealed with aluminum foil and kept at room temperature prior to analyses.

Preparation of protein isolate

This was prepared as described by Aremu *et al.,* (2007). One part of the defatted flour was dispersed in ten parts of distilled water (50 ºC), by stirring for 30 min. The pH of the dispersion was adjusted to pH 9.5 by drop wise addition of 0.1 M NaOH. The extraction was continued by occasional stirring for 6 h. The dispersion was centrifuged at 400 rpm for 30 min to separate the solid, aqueous and oil/emulsion phases. The aqueous supernatant was adjusted to pH 4.5 using 0.1 M HCl and was centrifuged and the precipitate neutralized, freeze dried and kept for analyses.

Formulation of composite flour

The flour used for biscuit production was obtained from blends of wheat flour, bambara groundnut flour and bambara groundnut protein isolate. The composite flour was obtained from response surface methodology (RSM) runs, which were evaluated from combination of wheat flour (WF), bambara groundnut flour (BF) and bambara protein isolate (BPI). Based on the crude protein and functional properties, the follarins ratios were selected WF: BF (85.0:15.0%, 76.2:23.8% and 52.5:47.5%) and labeled as WBF_1, WBF_2, WBF_3, respectively with WF: BPI (85.0:15.0%, 76.2:23.8% and 52.5:47.5%) as $WBPI_1$, $WBPI_2$ and $WBPI_3$ for blends of wheat flour and bambara protein isolate respectively. The 100% wheat flour biscuit was used as the control sample.

Production of biscuit

Bambara groundnut flour and protein isolate were weighed using a digital balance and blended together with wheat at different ratios using a Kenwood electronic blender (JT302N, England), to obtain composite flour. Other ingredients such as sugar, water, baking powder, salt, egg, flavour and butter were also added. After mixing, they were allowed to proof, cut into different shapes, arranged on a tray and subsequently baked in an oven at 150 ºC for 30 min prior to drying in the same oven at 50 ºC for 15 min. This was done by modifying Nwosu (2013) method. The samples were allowed to cool before packaging in a flexible material and kept till further use.

Analyses

Determination of amino acid

Amino acid compositions of the samples were determined on hydrolysates using amino acid analyser (Sykam-S7130). The method used was the ion exchange chromatography. Sample hydrolysates were prepared following the modified method of AOAC (2012). Each of the defatted samples was weighed (200 mg) into glass ampoule, 5 ml of 6 M HCl added and hydrolysed in an oven preset at 105 ± 5 °C for 22 h. Oxygen was expelled in the ampoule by passing nitrogen gas into it. Amino acid analysis was done by ion-exchange chromatography Spackman *et al*., (1958) using a Technicon Sequential Multisample Amino Acid Analyzer (Technicon Instruments Corporation, New York, USA). The period of analysis was 76 min, with a gas flow rate of 0.50 ml/min at 60 °C, and the reproducibility was ± 3%. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein.

Determination of nutritional qualities by calculation

Nutritional qualities were determined on the basis of the amino acid profile. The Essential Amino Acid Index (EAAI) was calculated using the method of Labuda *et al*. (1982) according to equation 1 below.

EAAI (Lys × Threo × Val × Meth × Isoleu × leu × Phynylal × Histi × Trypt)

(Lys × Threo × Val × Meth × Isoleu ×leu × Phynylal × Histi × Trypt)(Eqn 1)

where: (lysine × threonine...) interest sample and (lysine× threonine...)

content of the same amino acids in standard protein (%; egg or casein) respectively.

Nutritional indices of the samples were calculated using the equations 2 and 3 below:

Biological value was calculated according to Oser, (1959) cited by Mune-Mune *et al*., (2011) using the following equation:

BV = 1.09 × essential amino acid index (EAAI) – 11.7 (Eqn 2)

The Protein Efficiency Ratio (PER) were estimated according to the regression equations developed by Alsmeyer *et al*., (1974) cited by Mune-Mune *et al.,* (2011) as given below:

PER = –0.468 + 0.454 (LEU) – 0.105 (TYR) (Eqn 3)

Protein digestibility corrected amino acid score (PDCAAS)

PDCAAS is a measure of the protein quality of a food based on the requirement pattern for human beings (FAO, 2011). Amino acid scores for the nine (9) essential amino acids were calculated according to equations 4 and 5. These calculations were based on the requirement pattern for children aged 1 to 2 years.

$$\text{Amino acid score} = \frac{\text{mg of Amino Acid in 1 g test protein}}{\text{mg of Amino Acid in ref protein(egg)}(1-2\text{ year olds})}$$ (Eqn 4)

PDCAAS =True Protein Digestibility x Lysine score or limiting Amino Acid Score (Eqn 5)

Statistical Analysis: All analyses were carried out in triplicates and they were analysed using Analysis of Variance. Data obtained were subjected to statistical analysis (ANOVA) using Statistical Package for the Social Sciences, SPSS (version 16). Mean values were separated using Duncan's New Multiple Range (DNMR) test and significance difference was accepted at 5% confidence level ($P \leq 0.05$).

Results and Discussion

Amino Acid Composition of the Composite Flour and Biscuit

The amino acid compositions of the composite flour and biscuit are presented in Tables 1 and 2, respectively. According to Pellet and Young (1980), the nutritive value of proteins depends primarily on the capacity to satisfy the needs of nitrogen and essential amino acids. The levels of some of the essential amino acids in the composite flour and biscuit were within that of the "ideal protein" 3.8 g/100g (FAO, 1992).

The composite flour and biscuit had very similar amino acid patterns. Protein isolation by alkali at high pH has been shown to cause various cross-linkages between amino acids; the

Samples	WBF_1	WBF_2	WBF_3	$WBPI_1$	$WBPI_2$	$WBPI_3$	FAO *Adult	FAO* Children
Glycine	4.38^{ef}	4.82^{a}	4.46^{cd}	4.45^{cd}	4.58^{b}	4.38^{ef}	-	-
Alanine	3.57^{cd}	3.25^{f}	3.48^{e}	3.78^{b}	3.82^{a}	3.57^{cd}	-	-
Serine	3.84^{ef}	4.52^{bc}	4.77^{a}	4.52^{bc}	4.25^{d}	3.84^{ef}	-	-
Proline	3.74^{ef}	4.12^{abc}	4.12^{abc}	3.90^{d}	4.12^{abc}	3.74^{ef}	-	-
Aspartic	10.91^{e}	13.03^{a}	4.39^{f}	11.60^{cd}	12.03^{b}	11.71^{cd}	-	-
Cysteine	1.85^{b}	1.71^{cde}	1.71^{cde}	1.53^{f}	1.71^{cde}	1.88^{a}	-	-
Glutamic	19.35^{b}	14.87^{f}	17.87^{de}	17.90^{c}	17.87^{de}	20.74^{a}	-	-
Tyrosine	3.05^{d}	2.58^{ef}	8.58^{a}	3.30^{b}	2.52^{ef}	3.27^{c}	-	-
Arginine	6.29^{f}	8.17^{c}	4.42^{e}	8.56^{ab}	8.58^{ab}	6.61^{d}		
Phenylalanine	4.15^{df}	4.42^{ab}	2.52^{e}	4.16^{df}	4.42^{ab}	4.37^{c}	2.50	6.90
Histidine	1.91^{ef}	2.52^{a}	2.17^{bc}	2.03^{d}	2.17^{bc}	1.98^{ef}	-	1.00
Methionine	0.72^{ef}	0.88^{bcd}	0.82^{bcd}	0.85^{bcd}	0.95^{a}	0.74^{ef}	1.50	2.70
Valine	4.56^{d}	4.63^{c}	12.03^{a}	4.32^{e}	4.12^{f}	4.85^{b}	2.60	3.80
Tryptophan	0.82^{df}	0.95^{abc}	0.74^{e}	0.98^{abc}	0.98^{abc}	0.88^{df}	0.40	1.25
Threonine	3.68^{c}	3.50^{d}	4.12^{a}	3.02^{e}	3.50^{d}	3.91^{b}	1.50	3.70
Isoleucine	4.37^{bcd}	4.39^{bcd}	3.50^{f}	4.14^{e}	4.39^{bcd}	4.72^{a}	2.00	3.10
Leucine	7.67^{f}	8.23^{bcde}	8.23^{bcde}	8.43^{a}	8.23^{bcde}	8.24^{bcde}	3.90	7.30
Lysine	6.26^{f}	6.58^{bcde}	6.58^{bcde}	6.93^{a}	6.58^{bcde}	6.56^{bcde}	3.00	6.40

Key: WBF1: Wheat flour 85.0% and Bambara groundnut flour 15.0%, WBF2: Wheat flour 76.2% and Bambara groundnut flour 23.8%, WBF3: Wheat flour 52.5% and Bambara groundnut flour 47.5%, WBPI1: Wheat flour 85.0% and Bambara groundnut protein isolate 15.0%, WBPI2: Wheat flour 76.2% and Bambara groundnut protein isolate 23.8%, WBPI3: Wheat flour 52.5% and Bambara groundnut protein isolate 47.5%

Table 1: Amino Acid Composition (g/100g protein) of the Composite Flour

Amino acids	WF	$WBPI_1$	$WBPI_2$	$WBPI_3$	WBF_1	WBF_2	WBF_3	FAO *Adult	FAO *Children
Glycine	2.15^{e}	4.24^{cd}	4.27^{cd}	4.46^{a}	2.08^{f}	4.18^{d}	4.34^{b}	-	-
Alanine	2.49^{fg}	4.29^{e}	4.67^{c}	4.96^{a}	2.47^{fg}	4.79^{b}	4.37^{d}	-	-
Serine	2.86^{fg}	3.85^{de}	3.93^{bc}	4.09^{a}	2.87^{fg}	3.82^{de}	3.93^{bc}	-	-
Proline	1.67^{ef}	2.92^{d}	3.08^{c}	3.27^{a}	1.66^{ef}	3.08^{c}	3.13^{b}	-	-
Aspartic acid	6.56^{g}	8.15^{cd}	8.07^{e}	9.04^{a}	6.85^{f}	8.79^{b}	8.19^{cd}	-	-
Cysteine	0.29^{fg}	0.37^{e}	0.56^{cd}	0.69^{ab}	0.25^{fg}	0.53^{cd}	0.61^{ab}	-	-
Glutamic acid	9.89^{g}	11.23^{de}	11.39^{de}	12.33^{ab}	9.94^{f}	12.01^{ab}	11.51^{c}	-	-
Tyrosine	1.85^{fg}	3.13^{e}	3.29^{cd}	3.45^{a}	1.83^{fg}	3.24^{cd}	3.34^{b}	-	-
Arginine	3.83^{g}	6.11^{bcd}	6.13^{bcd}	6.32^{a}	3.98^{f}	6.03^{e}	6.15^{bcd}	-	-
Phenylalanine	2.11^{fg}	4.09^{e}	4.12^{cd}	4.39^{a}	2.13^{fg}	4.12^{cd}	4.21^{b}	2.50	6.90
Histidine	2.18^{fg}	3.81^{e}	4.02^{abc}	4.08^{abc}	2.17^{fg}	3.91^{d}	4.05^{abc}	-	1.00
Methionine	1.34^{f}	2.78^{e}	2.86^{bcd}	3.09^{a}	1.29^{g}	2.84^{bcd}	2.89^{bcd}	1.50	2.70
Valine	3.95^{f}	5.16^{bc}	5.09^{de}	5.37^{a}	3.88^{g}	5.08^{de}	5.13^{bv}	2.60	3.80
Tryptophan	3.96^{f}	5.11^{bcd}	5.16^{bcd}	5.38^{a}	3.81^{g}	5.09^{e}	5.17^{bcd}	0.40	1.25
Threonine	2.27^{fg}	3.66^{e}	3.91^{cd}	4.18^{a}	2.25^{fg}	3.97^{cd}	4.06^{b}	1.50	3.70
Isoleucine	1.95^{fg}	3.21^{bc}	3.18^{de}	3.67^{a}	1.92^{fg}	3.27^{bc}	3.19^{de}	2.00	3.10
Leucine	4.28^{g}	5.92^{bcde}	5.92^{bcde}	6.24^{a}	4.34^{f}	5.92^{bcde}	5.94^{bcde}	3.90	7.30
Lysine	3.02^{fg}	6.16^{de}	6.19^{de}	6.88^{a}	3.07^{fg}	6.28^{bc}	6.25^{bc}	3.00	6.40

Key: WBF1: Wheat flour 85.0% and bambara groundnut flour 15.0%, WBF2: Wheat flour 76.2% and bambara groundnut flour 23.8%, WBF3: Wheat flour 52.5% and bambara groundnut flour 47.5%,WBPI1: Wheat flour 85.0% and bambara groundnut protein isolate 15.0%,WBPI2: Wheat flour 76.2% and bambara groundnut protein isolate 23.8%,WBPI3: Wheat flour 52.5% and bambara groundnut protein isolate 47.5%,WF: 100% Wheat flour

Table 2: Amino Acid Composition (g/100g protein) of Biscuit

cross-linkages of major concern are lysinoalanine formation from lysine and dehydroalanine through degradation of cystine or serine, because it reduces protein digestibility. Composite flour from WBF_1 – $WBPI_3$ contained high levels of leucine which ranged between 7.67 – 8.43 g/100g which were above the values for adult 3.90 g/100g and children 7.30 g/100g FAO, (2007), Arginine ranged between 4.42 – 8.58 g/100g and lysine ranged between 6.26 – 6.93 g/10g with 3.00 g/100g for adult and 6.40 g/100g for children (FAO, 2007). Biscuit from WF-$WBPI_3$ contained leucine which ranged between 4.28 – 6.24 g/100g which were above value for adult 3.90 g/100g and and lower than that of children 7.30 g/100g (FAO, 2007). Arginine ranged between 3.83-6.32 g/100g and lysine ranged between 3.02 – 6.88 g/100g with 3.00 g/100g for adult and 6.40 g/100g for children (FAO, 2007). The high lysine content of the composite flour and biscuit protein is a very important nutritional attribute that makes the legume a good supplementary protein to cereals that are known to be deficient in lysine.

The combined summary of the compositions of the composite flour and biscuit (Tables 3 and 4, respectively) shows content of sulphur-containing amino acids, such as methionine, in the composite flour to be low 0.72 – 0.95 g/100g compared to 1.50 – 2.70 g/100g (FAO, 2007) while that of the biscuit ranged between 1.29 – 3.09 g/100g which falls within 1.50 – 2.70 g/100g (FAO, 2007). Methionine and cysteine are considered to be limiting amino acids in *Phaseolus* beans (Sosulski and McCurdy, 1987; Paredes-Lopez *et al.*, 1991). The low level of methionine in the flour is similar to other legume seed protein reported by previous researchers (Sosulski and McCurdy, 1987; Paredes-Lopez *et al.*, 1991). Nutritionally, the percentage ratios of TEAA to TAA in the composite flour and biscuit samples were all above the values considered to be adequate for ideal protein for infants (39%), children (26%) and adults (11%) (FAO, 2007) as presented in Tables 3 and 4, respectively.

Predicted Nutrtional Quality	WBF_1	WBF_2	WBF_3	$WBPI_1$	$WBPI_2$	$WBPI_3$
ΣNEAAs	57.00^{de}	57.06^{de}	53.81^{f}	59.54^{bc}	59.48^{bc}	61.11^{a}
TAA	91.14^{f}	93.16^{e}	94.52^{bcd}	94.40^{bcd}	94.82^{bcd}	97.35^{a}
TEAA/TAA%	37.46^{cde}	38.75^{b}	43.07^{a}	36.93^{f}	37.27^{cde}	37.23^{cde}
TNEAA/TAA%	62.54^{bcd}	61.25^{e}	56.93^{f}	63.07^{a}	62.73^{bcd}	62.77^{bcd}
TEAA/TNEAA	0.69^{abc}	0.63^{abc}	0.64^{abc}	0.59^{def}	0.59^{def}	0.59^{def}
ΣSAAs (Meth+Cys)	2.57^{cde}	2.59^{cde}	2.53^{cde}	2.38^{f}	2.66^{ab}	2.62^{ab}
ΣArAAs (Phe+Tyr)	7.20^{d}	7.00^{e}	11.10^{a}	7.46^{c}	6.94^{f}	7.64^{b}
P-PER	2.40^{f}	2.64^{bc}	2.70^{a}	2.59^{de}	2.62^{bc}	2.55^{de}
EAAI (%)	57.16^{f}	61.78^{b}	60.92^{cd}	66.35^{a}	60.81^{cd}	60.11^{e}
P-BV (%)	50.60^{f}	55.64^{b}	54.70^{cd}	60.63^{a}	54.58^{cd}	53.82^{e}

EAA- essential amino acid, NEAA- non essential amino acid, TAA- total amino acid, TEAA- total essential amino acid, SAA-sulphur containing amino acids (methionine and cysteine), EAAI-essential amino acid index, BV- biological value, PER- protein efficiency ratio, TNEAA- total non essential amino acid, ArAA- aromatic amino acid (Phenylalanine and Tryptophan)

Key: WBF_1: Wheat flour 85.0% and bambara groundnut flour 15.0%,
WBF_2: Wheat flour 76.2% and bambara groundnut flour 23.8%,
WBF_3: Wheat flour 52.5% and bambara groundnut flour 47.5%,
$WBPI_1$: Wheat flour 85.0% and bambara groundnut protein isolate 15.0%,
$WBPI_2$: Wheat flour 76.2% and bambara groundnut protein isolate 23.8%,
$WBPI_3$: Wheat flour 52.5% and bambara groundnut protein isolate 47.5%

Table 3: Summary of Amino Acid and Nutritional Indices of the Composite Flour (g/100g protein)

Predicted Nutritional Qualities	WF	$WBPI_1$	$WBPI_2$	$WBPI_3$	WBF_1	WBF_2	WBF_3
ΣEAAs+ Histidine	25.06^{f}	39.9^{e}	40.45^{bcd}	43.28^{a}	24.86^{g}	40.48^{bcd}	40.89^{bcd}
ΣNEAAs	31.59^{fg}	44.29^{e}	45.39^{cd}	48.61^{a}	31.93^{fg}	46.47^{b}	45.57^{cd}
TAAs	56.65^{fg}	84.19^{e}	85.84^{d}	91.89^{a}	56.79^{fg}	86.95^{bc}	86.46^{bc}
TEAAs/TAAs%	55.76^{a}	52.61^{bc}	47.12^{de}	47.09^{de}	43.78^{g}	46.56^{f}	52.71^{bc}
TNEAAs/TAAs%	55.76^{c}	84.19^{a}	52.88^{g}	52.90^{de}	56.22^{b}	53.44^{f}	52.71^{de}
TEAAs/TNEAAs	0.79^{fg}	0.90^{a}	0.89^{bcde}	0.89^{bcde}	0.78^{fg}	0.87^{bcde}	0.89^{bcde}
ΣSAAs (Meth+Cys)	1.63^{f}	3.15^{e}	3.42^{c}	3.78^{a}	1.54^{g}	3.37^{d}	3.50^{b}
ΣArAAs (Phe+Tyr)	6.07^{f}	9.20^{cde}	9.28^{cde}	9.77^{a}	5.94^{g}	9.21^{cde}	9.38^{b}
Arginine/Lysine	1.27^{ab}	0.99^{cdef}	0.99^{cdef}	0.92^{g}	1.29^{ab}	0.96^{cdef}	0.98^{cdef}
BCAAs	10.18^{fg}	14.29^{bcd}	14.19^{e}	15.28^{a}	10.14^{fg}	14.27^{bcd}	14.26^{bcd}
P-PER	1.70^{g}	2.69^{e}	2.85^{bc}	3.05^{a}	2.50^{f}	2.71^{d}	2.88^{bc}
EAAI (%)	37.06^{g}	70.84^{c}	72.13^{b}	76.49^{a}	61.04^{f}	62.13^{de}	62.78^{de}
BV (%)	47.17^{g}	58.42^{de}	62.79^{b}	66.99^{a}	56.11^{f}	58.32^{de}	61.84^{c}

EAA- essential amino acid, NEAA- non essential amino acid, TAA- total amino acid, TEAA- total essential amino acid, SAA-sulphur containing amino acids (methionine and cysteine), EAAI-essential amino acid index, BV- biological value, PER- protein efficiency ratio, TNEAA- total non essential amino acid, ArAA- aromatic amino acid (Phenylalanine and Tryptophan), BCAA- basic amino acid

Table 4: Summary of Amino Acid and Nutritional Indices of Biscuit (g/100g protein)

Key: WBF_1: wheat flour 85.0% and bambara groundnut flour 15.0%, WBF_2: wheat flour 76.2% and bambara groundnut flour 23.8%, WBF_3: wheat flour 52.5% and bambara groundnut flour 47.5%, $WBPI_1$: wheat flour 85.0% andbambara groundnut protein isolate 15.0%, $WBPI_2$: wheat flour 76.2% and bambara groundnut protein isolate 23.8%, $WBPI_3$: wheat flour 52.5% and bambara groundnut protein isolate 47.5%, WF: 100% wheat flour

Hence, the essential amino acids in the composite flour and biscuit may adequately support growth and development in infants, children and adults. The protein efficiency ratio – PER (chemical) which is used as a good estimate of protein quality (Pastor-Cavada *et al*., 2009) of the composite flour and biscuit is presented in Tables 3 and 4, respectively. The calculated PER ranged between 2.40 – 2.70 which is an indication of good quality protein. These values compare favourably well with previously reported PER of plant proteins, 2.1 (soy) and 1.5 (wheat); animal protein 2.9 (beef), 3.8 (egg) and 3.1 (cow's milk) (FAO/WHO, 1998; European dairy Association). However, considering the low biological values (BV) of the composite flour and biscuit which may be due to denaturation by the processing conditions they were subjected to, this suggests some of the amino acids may not be available for body use.

Conclusion

Addition of bambara groundnut flour resulted in increase in the nutritional composition of the flour and biscuit samples including protein content and quality indices and its ratios. All of these increased as the supplementation level increased. The amino acid results indicated that, the new products are superior with respect to arginine, leucine, phenylalanine, threonine and valine when compared to FAO/WHO reference pattern.

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