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Evaluation of Amino Acid Profile and Protein Quality of Biscuits Produced from Wheat (*Triticum aestivum*) and Red Kidney Bean (*Phaseolus vulgaris L*) Flour Blends

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Abstract

This study was carried out to evaluate the amino acid profile and protein quality of biscuit produced from wheat (*Triticum aestivum)* and red kidney bean *(Phaseolus vulgaris L*) flour blends. The red kidney bean seed were sorted, cleaned, boiled, drained, oven dried, cracked, dehulled, milled, sieved to obtain the red kidney bean flour. The wheat flour was blended with red kidney bean flour in the ratio of 100:0 (sample A), 90:10 (sample B), 80:20 (sample C), 70:30 (sample D) and 60:40 (sample E) for wheat and red kidney beans respectively and use for the production of biscuits. Biscuits were analyzed for amino acid profile and the protein quality of the biscuits were also determined using standard methods. Casein diet (sample AA) and nitrogen free diet (sample F) where the positive and negative control for the protein quality study. A total of thirty-five (35) weaning albino rats were used for the study. The diets were subjected to amino acid profile and protein quality. The data obtained were subjected to statistical analysis. The results of the amino acid profile were glutamic, tyrosine, arginine, aspartic acid and lysine, had the values, 8.62 g/100g, 6.55 g/100g, 5.43 g/100g, 5.28 g/100g and 5.23 g/100g respectively. The result of the protein quality showed that about 98% of the nitrogen consumed by all the five groups of rats fed with the biscuit diets were absorbed and retained. The biological value ranged from 95.59±0.23 to 98.57±0.46%, the net protein utilization ranged from 90.91±0.17 to 95.04±0.65%, the true protein digestibility ranged from 49.43±6.89 to 86.04±3.04% and the protein efficiency ratio ranged from 1.38±0.31 to 2.54±0.62. Conclusively, this study showed that substituting biscuit with red kidney beans flour increased the amino acid profile and improved the protein quality of the biscuit.

Keywords: Amino acid profile, Protein quality, Biscuit, Red kidney beans.

1. Introduction

Snack is a portion of food, smaller than a regular meal generally eaten between meals. Snacks come in a variety of food including baked and packaged snacks food, examples include cookies, biscuits, doughnut, ice cream, peanut, shortbread, tea cake, etc (Onwurafor *et al.,* 2019). The demand for biscuit as popular snacks from children and adolescents at school during break time and other occasions like birthday cannot be overemphasized. Biscuit is produced by mixing various ingredients like wheat flour, fat sweetener and water to form dough and baked in the oven (Adegbanke *et al.,* 2020). Protein malnutrition is common in baked products because our bodies cannot produce all the essential amino acids (Abimbola *et al.,* 2020), wheat flour is deficient in lysine, an essential amino acid and so the need to blend with red kidney bean flour. Wheat provides carbohydrate in form of starch with a percentage of 80-85 which is high calories value (Mamat and Hill, 2018). Wheat contains good quantity of protein of about 12.01%. Its protein is good source of amino acid which is high in leucine, wheat can be improved by supplementation with legume/pulse (Oyet and Chibor, 2020). Wheat is high in gluten which is the reason why it is used in the bread and biscuit industries.

Red kidney beans (*Phaseolus vulgaris L*), a grain legume, is one of the most widely cultivated and consumer food legumes (Noah and Adedeji, 2021). It is one of the tropical legumes that can be used to improve the diets of millions of people, both low- and medium-income earners who cannot afford expensive animal protein because of their high prices in Nigeria. It is a vital source of protein (22.7%), B-vitamins and minerals (Noah and Adedeji, 2021).

Proteins play a crucial role in the growth, maintenance and physiological functions of the human body (Vignati *et al.,* 2024). All amino acids are important in the synthesis and functioning of muscles and organs, as well as in enzymes, hormones and the immune system (Duan *et al.,* 2015). To meet the metabolic demand and to assure proper functioning of the human body, consumption of adequate amounts of protein is thus essential to meet both total nitrogen and indispensable amino acid requirements (Vignati *et al.,* 2024). The protein quality of the biscuit from the blends of wheat and kidney bean flours needs to be ascertained to know whether they meet up with the recommended nutrient need of protein for children between two to five years. Therefore, this study intends to produce biscuits from flours obtained from wheat and kidney bean and evaluate the protein quality.

2. Materials and Methods

2.1 Procurement of Raw Materials

The raw materials which were commercial wheat flour, red kidney beans, sugar, margarine, salt, baking powder, flavor and milk were bought from Ogbete main market in Enugu North Local Government Area of Enugu State, Nigeria.

2.2 Preparation of Kidney Bean Flour

The method described by Chaudhary and Sharma (2013) with slight modifications was followed in the preparation of red kidney bean flour. The beans were thoroughly cleaned, to remove extraneous materials and dirt, blanched in hot water (1:5w/v) at 100^0C for 30 min, drained, oven dried at 55^0C for 6 hours, dehulled, winnowed, milled, sieved using sieve mesh of 60mm size, packaged in an air tight container, for subsequent use.

2.3 Formulation of Flour Blends

To formulate different flour blends a Completely Randomized Design (CRD) was adopted for this study design. In this design, red kidney bean and wheat flours were blended together at different ratios and used to produce different samples of biscuits. The **w**heat and red kidney bean flours were thoroughly mixed together in the ratios of A-100:0, B-90:10, C-80:20, D-70:30 and E-60:40 respectively in a mixer to produce composite flours. The composite flours produced were packaged separately and kept for biscuit production.

2.4 Preparation of Biscuits.

The method described by Adegbanke *et al.,* (2020) was adopted in the biscuit production with modification in the baking temperature and time. The biscuit formulation comprised of 200g flour, 118g sugar, 110g margarine, 50mL whole egg, 25mL water, 50 mL milk, 3g salt, 3g baking powder and 3g vanilla flavour. The composite flours were blended with other baking ingredients in a mixer, kneaded for 12 min with a kneading machine into a constant dough. The resulting dough was cut into uniform sizes and passed through a series of moulding, shaping and stamping. The stamped dough was baked in an oven for 30 min at 150^{0}C, the products were allowed to cool and were subsequently packaged with a cellophane wrapper and stored at room temperature for further use.

2.5 Amino Acid Determination

Determination of amino acid composition of the samples were determined on hydrolysates using amino acid analyzer (Sykam-S7130). The method used was the ion exchange chromatography. Sample hydrolysates were prepared following the modified method of AOAC (2010). Each of the defatted samples was weighed (200 mg) into glass ampoule, 5 mL of 6 M HCL added and hydrolyzed in an oven preset at 105 ± 5^{o}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 Singh *et al.* (2014) using a Technicon Sequential Multisampling Amino Acid Analyzer (Technicon Instruments Corporation, New York, USA). The period of analysis was 76 minutes, with a gas flow rate of 0.50 mL/min at 60^{o}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.

2.6 Diet Formulation

Seven diets were formulated. These include casein diet (AA), 100:0 wheat flour to red kidney bean flour biscuit (A1), 90:10 wheat flour to red kidney bean flour biscuit (B1), 80:20 wheat flour to red kidney bean flour biscuit (C1), 70:30 wheat flour to red kidney bean flour biscuit (D1), 60:40 wheat flour to red kidney bean flour biscuit (E1) and Nitrogen free diet (F). The milk diet was the reference. The biscuits were pulverised and the crude protein content of the biscuit flours determined as described by AOAC (2010) and then used for the formulation of test diets. The diets were prepared by incorporating sucrose, corn starch, vegetable oil, vitamin mix, mineral mix to obtain the required 1000g by volume as shown in table 1. The dried ingredients for each diet were measured and small quantity of water added until a homogenous mixture was formed. The mixture was used to form pellets for feeding the rats. The amount for the diet were calculated using the formula;

$$10\%\ \text{Protein Content} = \frac{3.2\ x\ 100}{\%\ weight\ of\ sample}$$

The diet was formulated using AIN-93G (American Institute of Nutrition) method of growth, pregnancy and lactating phases in laboratory rats (Klurfeld *et al.,* 2021).

2.7 Animal and Housing

Thirty-five weaning male albino rats from the same colony weighing about 40-60 g were purchased from the veterinary medicine Department, University of Nigeria Nsukka. The rats were housed in individual metabolism cages equipped to separate faeces from urine on a base tray. All procedure using animal in this investigation were followed in accordance with ethical standard of European Union guidelines for animal experimentation (Dir 86/609/EEC) and were approved by Industrial animal care committee, University of Nigeria Nsukka.

2.8 Growth and Maintenance Study

After a 7-day acclimatization period, the rats were weighed prior to access to their respective diets. The casein and the test diets were fed 15 g each to the rats daily for 21-day growth period. During the study, food intake was measured on daily basis for day 21, carmine red was added to each diet to mark the beginning of nitrogen balance (N) and end of nitrogen balance. On day 22, all red faeces were retained and the black ones discarded. Black faeces collected after day 23 were discarded until day 28 when another carmine red was added to mark the end of N balance period. On day 29, all the red faeces were discarded. The faeces were sun dried for 8 h, weighed, ground and stored in deep freezer for analysis. The rats were sacrificed on day 29. The liver was removed, weighed and analyzed. The proportion of food nitrogen that is absorbed was calculated thus:

$$A= \frac{I\ (F- Fk)x\ I}{1}$$

Where A= absorbed nitrogen
F= faecal nitrogen
Fk= metabolic nitrogen
I= nitrogen intake

2.9 Determination of Protein Efficiency Ratio, Biological Value, True Protein Digestibility and Net Protein Utilization

The weight gain and feed intake for each group of the rat was used to estimate protein efficiency ratio (PER) as described by FAO/WHO/UNU (2007). The biological value was obtained by estimating the retained nitrogen over total nitrogen intake, with corrections for faecal and urinary losses of each group of the rat (FAO/WHO/UNU, 2007). True protein digestibility (TPD) was determined via diet consumption, fecal nitrogen loss by fecal nitrogen, and metabolic nitrogen loss by determining fecal nitrogen in rats consuming a protein free diet as described by FAO (2013). The values obtained for feacal, digested, urinary and retained or absorbed nitrogen from each group of rats was used in calculating net protein utilization (NPU) as described by (FAO/WHO/UNU, 2007).

2.10 Experimental Design

A Completely Randomized Design (CRD) was adopted for this study. The different diets were the treatment and rat were the replicates.

2.11 Statistical Analysis

Amino acid analysis was determined in triplicate while the rat study was determined in a group of five rats each and were subjected to analysis. The data generated were analyzed using Statistical Package for the Social Sciences (SPSS Version 20) software to determine the mean, standard deviation and analysis of variance (ANOVA). Mean values were separated using Duncan's New multiple range test and significance difference was accepted at 95% confidence level ($P < 0.05$).

3. Results

Table 1: Composition of experimental diet (g/kg)

Diet Ingredients (g/Kg)	AA	A1	B1	C1	D1	E1	F
Casein	309.28	0	0	0	0	0	0
Samples	0	682.12	659.74	641.14	635.94	628.79	0
Corn starch	240.98	0	0	0	0	0	395.62
Sucrose	240.98	109.12	131.50	150.10	155.30	162.45	395.62
Vegetable oil	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Vitamin mix	48.56	48.56	48.56	48.56	48.56	48.56	48.56
Mineral Mix	60.20	60.20	60.20	60.20	60.20	60.20	60.20
Total	1000	1000	1000	1000	1000	1000	1000

AA-Casein diet, A1 – Biscuit from 100% wheat flour, B1 –Biscuit made with 90% wheat flour and 10% red kidney bean flour, C1 –Biscuit made with 80% wheat flour and 20% red kidney bean flour, Sample D1- Biscuit made with 70% wheat flour and 30% red kidney bean flour and E1 – Biscuit made with 60% wheat flour and 40% red kidney bean flour, F-Nitrogen free diet.

Table 2: Amino Acid Profile (g/100g protein) of the Biscuit Samples

Samples	A	B	C	D	E	FAO/WHO (2-5 Years)
Essential Amino Acids						
Arginine	1.05^{d}±0.38	2.40^{c}±0.42	3.09^{bc}±0.58	4.06^{b}±0.58	5.43^{a}±0.95	2.5
Histidine	0.07^{c}±0.01	0.20^{c}±0.19	0.93^{b}±0.74	1.16^{b}±0.09	2.24^{a}±0.25	1.9
Threonine	2.02^{b}±0.61	2.26^{b}±1.75	2.99^{ab}±0.31	3.50^{ab}±0.34	4.09^{a}±0.33	3.4
Valine	2.43^{d}±0.10	2.56^{d}±0.05	3.13^{c}±0.35	3.99^{b}±0.15	4.76^{a}±0.50	3.5
Isoleucine	2.34^{b}±1.21	2.98^{ab}±1.01	3.60^{ab}±0.87	4.12^{ab}±0.70	4.62^{a}±0.92	2.8
Lysine	2.39^{c}±0.65	3.39^{b}±0.50	3.81^{b}±0.21	5.06^{a}±0.77	5.23^{a}±0.38	5.8
Methionine	1.36^{c}±0.29	2.03^{bc}±0.40	2.29^{bc}±0.77	2.49^{b}±0.31	3.64^{a}±0.80	2.2
Tryptophan	1.15^{c}±0.21	2.43^{b}±0.76	3.08^{ab}±0.35	3.26^{ab}±0.35	3.94^{a}±0.41	1.1
Leucine	0.93^{c}±0.27	1.22^{c}±0.27	1.91^{b}±0.32	2.44^{b}±0.27	3.12^{a}±0.43	6.6
Phenylalanine	1.99^{c}±0.39	2.26^{c}±0.35	2.76^{bc}±0.29	3.19^{ab}±0.25	3.72^{a}±0.72	2.8
TEAA	20.91^{e}±0.37	28.81^{d}±0.42	36.31^{c}±0.49	42.85^{b}±0.38	53.73^{a}±0.57	
Non-Essential Amino Acids						
Cysteine	0.46^{d}±0.16	1.52^{c}±0.80	2.28^{bc}±0.61	3.12^{b}±0.49	4.48^{a}±0.35	
Glycine	0.32^{d}±0.10	1.06^{d}±0.60	1.97^{c}±0.44	2.43^{b}±0.54	3.91^{a}±0.43	
Serine	2.30^{b}±0.53	2.83^{b}±0.42	4.23^{a}±0.87	4.58^{a}±0.45	4.78^{a}±0.33	
Proline	2.10^{c}±0.44	2.78^{bc}±0.28	3.09^{b}±041	3.46^{ab}±0.53	4.22^{a}±0.42	
Aspartic acid	0.74^{d}±0.27	1.53^{cd}±0.78	2.29^{bc}±0.55	3.52^{b}±0.89	5.28^{a}±1.02	
Glutamic	3.71^{c}±1.36	4.78^{c}±0.48	6.40^{b}±1.11	7.85^{a}±0.61	8.68^{a}±0.42	
Tyrosine	2.42^{c}±0.95	3.67^{bc}±1.02	4.48^{b}±0.74	4.84^{b}±0.24	6.55^{a}±1.03	
Alanine	2.04^{d}±0.08	2.41^{cd}±0.56	3.31^{bc}±0.63	4.10^{b}±0.47	5.15^{a}±0.73	
TAA	36.05^{e}±0.45	51.79^{d}±0.59	67.45^{c}±0.56	80.81^{b}±0.45	102.21^{a}±0.58	

Values are mean ± standard deviation of triplicate samples. Mean values bearing different superscript in the same row differ significantly (P<0.05). * = Essential Amino Acid. A – Biscuit from 100% wheat flour, B –Biscuit made with 90% wheat flour and 10% red kidney bean flour, C –Biscuit made with 80% wheat flour and 20% red kidney bean flour, Sample D- Biscuit made with 70% wheat flour and 30% red kidney bean flour and E – Biscuit made with 60% wheat flour and 40% red kidney bean flour, TEAA- Total Essential Amino Acid, TAA- Total Amino Acid.

Table 3: Protein Quality of rats fed with casein and biscuit containing test diets.

Diets	Protein efficiency ratio (PER)	Biological Value (BV) %	Net Protein Utilization (NPU) %	True Protein Digestibility (TPD) %
AA	2.71^{a}±5.64	96.29^{a}±0.10	94.59^{a}±0.40	92.09^{a}±2.55
A1	1.38^{b}±0.31	68.27^{e}±0.46	65.63^{f}±0.55	49.43^{f}±6.89
B1	2.07^{ab}±0.72	74.52^{d}±0.80	70.46^{e}±1.75	63.03^{e}±3.81
C1	2.39^{a}±0.61	86.77^{c}±0.74	81.70^{d}±1.88	70.80^{d}±4.03
D1	2.47^{a}±1.13	92.51^{b}±0.70	87.23^{c}±1.67	80.92^{c}±3.64
E1	2.54^{a}±0.62	92.46^{b}±0.10	88.09^{b}±0.11	86.04^{b}±3.04
F	ND	ND	ND	ND

Values are mean ± standard deviation of seven (7) rat groups. Mean values bearing different superscript in the same row differ (P<0.05) significantly. AA-Casein diet, A1 – Rat fed diet of 100% wheat, B1 –Rat fed diet of 90% wheat and 10% red kidney bean, C1 –Rat fed diet of 80% wheat and 20% red kidney bean, D1 -Rat fed diet of 70% wheat and 30% red kidney bean and E1 – Rat fed diet of 60% wheat and 40% red kidney bean, F-Nitrogen free diet.

4. Discussion

The adequacy of a dietary protein to meet the indispensable amino acid (IAA) requirements of humans is often considered the basis of expression of protein quality. Several commonly used principles of expressing protein quality are based on the ability of a protein source to supply sufficient IAA for a specific target group (FAO 2013; Adhikari *et al.,* 2022), For the essential amino acids, histidine of wheat-red kidney biscuit ranged from 0.07 to 2.24 g/100g. The value of histidine in the biscuit samples increased with an increase in the substitution of red kidney bean flour. The histidine content of sample E (60% wheat flour and 40% red kidney flour) was significantly ($p<0.05$) high compared to all the other samples. The FAO requirements for histidine for children between the ages of 2 to 5 years is 1.9 g/100g (FAO, 2007) and in all the samples, sample E was the only sample which attained and surpassed that requirement. The threonine of wheat-red kidney biscuit ranged from 2.02 to 4.09 g/100g. The value of threonine increased with an increase in the substitution of red kidney bean flour. Threonine content of sample E (4.09 g/100g) was significantly ($p<0.05$) high compared to all the other samples. The requirement for threonine by FAO for children between the ages of 2 to 5 years is 3.4 and sample D (70% wheat flour and 30% red kidney bean flour) and E which having 3.5 and 4.09 g/100g, respectively met the requirement.

The valine content of wheat-red kidney biscuit ranged from 2.43 to 4.76 g/100g. The value of valine increased with an increase in the substitution of red kidney bean flour. Valine content of sample E was significantly ($p<0.05$) high compared to all the other samples. The valine FAO requirements (FAO, 2007) for children between the ages of 2 to 5 years is 3.5 g/100g and consumption of 100% of samples D and E which had 3.99 and 4.76 g/100g respectively was capable to meet the requirement. The isoleucine content of wheat-red kidney biscuit ranged from 2.34 to 4.62 g/100g. The value of isoleucine increased with an increase in the substitution of red kidney bean flour. Isoleucine content of sample E (60% wheat flour and 40% red kidney flour) which is 4.62 g/100g was significantly ($p<0.05$) high compared to all the other samples. The FAO requirements for isoleucine for children between the ages of 2 to 5 years is 2.8 g/100g (FAO, 2007) and samples B, C, D and E meets the requirement.

The lysine of wheat-red kidney biscuit ranged from 2.39 to 5.23 g/100g. The value of lysine increased with an increase in the substitution of red kidney bean flour. The Lysine content of samples D (70% wheat flour and 30% red kidney flour) and E was not significantly ($p>0.05$) different. Also, samples, B (90% wheat flour and 10% red kidney flour) and C (80% wheat flour and 20% red kidney flour) was not significantly ($p>0.05$) different while sample A (100% wheat flour) was significantly ($p<0.05$) different from all the other sample and had the least lysine content. The requirement for lysine by FAO for children between the ages of 2 to 5 years is 5.8 g/100g and none of the samples met up to the requirement. Lysine is essential for children as it is critical for bone formation. It is involved in hormone production and lowers serum triglyceride levels (Oyet and Chibor, 2020). The Lysine content of between 2.39 and 5.23g/100g was lower than that stipulated for children which is 5.8 g/100g (FAO, 2007). The high lysine content of the biscuit is a very important nutritional attribute that makes the legume a good supplementary protein to cereals that are known to be deficient in lysine (Adegbanke *et al.,* 2024).

The Methionine content of wheat-red kidney biscuit ranged from 1.36 to 3.64 g/100g. The value of methionine increased with an increase in the substitution of red kidney bean flour. Methionine content of sample E (60% wheat flour and 40% red kidney flour) was significantly ($p<0.05$) high compared to all the other samples. The requirement for methionine by FAO for children between the ages of 2 to 5 years is 2.2 g/100g (FAO, 2007) and samples C (80% wheat flour and 20% red kidney bean flour), D (70% wheat flour and 30% red kidney flour) and E meets the requirement. The Tryptophan content of wheat-red kidney biscuit ranged from 1.15 to 3.94 g/100g. The value of tryptophan increased with an increase in the substitution of red kidney bean flour. The requirement for tryptophan by FAO for children between the ages of 2 to 5 years is 1.1 g/100g (FAO, 2007) and consumption of 100% of samples A, B, C, D and E was capable to meet up with the requirement. Arginine content of wheat-red kidney biscuit ranged from 1.05 to 5.43 g/100g. The value of arginine increased with an increase in the substitution of red kidney bean flour. Arginine content of sample E was significantly ($p<0.05$) high compared to all the other samples. The requirement for arginine by FAO for children between the ages of 2 to 5 years is 2.5 (FAO, 2007) and samples C (80% wheat flour and 20% red kidney bean flour), D and E meets the requirement. Arginine is indispensable only in high anabolic activity, such as the tissue growth of childhood and has an important role in immune function, and the release of hormones (Huang *et al.,* 2017).

The leucine content of wheat-red kidney biscuit ranged from 0.93 to 3.12 g/100g. The value of leucine increased with an increase in the substitution of red kidney bean flour. The requirement for leucine by FAO for children between the ages of 2 to 5 years is 6.6 g/100g (FAO, 2007) and none of the biscuit samples met the requirement. The Phenylalanine content of wheat-red kidney biscuit ranged from 1.99 to 3.72 g/100g. The value of Phenylalanine increased with an increase in the substitution of red kidney bean flour. Phenylalanine content of sample E (60% wheat flour and 40% red kidney flour) was significantly ($p<0.05$) high compared to all the other samples. The requirement for Phenylalanine by FAO for children between the ages of 2 to 5 years is 2.8 g/100g and samples D and E met the requirement. 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, 2013). The essential amino acids in the biscuits may adequately support growth and development in children and adults.

For non-essential amino acids, cysteine ranged of wheat-red kidney biscuit from 0.46 to 4.48 g/100g. The value of cysteine increased with an increase in the substitution of red kidney bean flour. Cysteine content of sample E (4.48 g/100g) was significantly ($p<0.05$) high compared to all the other samples. Cysteine is a precursor to glutathione (GSH), a powerful antioxidant that fights free radicals and about 30% of the dietary requirement for methionine can be replaced by cysteine (FAO/WHO/UNU, 2007). The glycine of wheat-red kidney biscuit ranged from 0.32 to 3.91 g/100g. The value of glycine increased with an increase in the substitution of red kidney bean flour. Glycine content of sample E (3.91 g/100g) was significantly ($p<0.05$) high compared to all the other samples. Glycine is a precursor of proteins, a biosynthetic intermediate and a neurotransmitter (Huang *et al.,* 2017).

The serine of wheat-red kidney biscuit ranged from 2.30 to 4.78 g/100g. The value of serine in the biscuit samples increased with an increase in the substitution of red kidney bean flour. Serine content of samples C, D and E (4.78 g/100g) were significantly ($p<0.05$) high than samples A and B. Serine is significant in metabolism because it is involved in the synthesis of purines and pyrimidines (Huang *et al.,* 2017). Serine is the precursor of some amino acids such as glycine and cysteine (Huang *et al.,* 2017). The proline of wheat-red kidney biscuit ranged from 2.10 to 4.22 g/100g. The value of proline increased with an increase in the substitution of red kidney bean flour. Proline content of sample E (4.22 g/100g) was significantly ($p<0.05$) higher than all the other samples. The principal function of proline is related to collagen structure, function and neurological function (Duan *et al.,* 2015).

The aspartic acid of wheat-red kidney biscuit ranged from 0.74 to 5.28 g/100g. The value of aspartic acid increased with an increase in the substitution of red kidney bean flour. Aspartic acid content of sample E (5.28 g/100g) was significantly ($p<0.05$) high compared to all the other samples. Aspartic acid is found in the neuroendocrine tissues of invertebrates and vertebrates and is involved in the development of the human nervous system (Huang *et al.,* 2017). The glutamic acid of wheat-red kidney biscuit ranged from 3.71 to 8.68 g/100g. The value of glutamic acid increased with an increase in the substitution of red kidney bean flour. Glutamic acid content of samples D and E (8.68 g/100g) was significantly ($p<0.05$) high compared to all the other samples. Glutamic acid is associated with healthy brain development and function in human body (Huang *et al.,* 2017).

The tyrosine content of wheat-red kidney biscuit ranged from 2.42 to 6.55 g/100 g. The value of tyrosine content increased with an increase in the substitution of red kidney bean flour in the biscuit. Tyrosine content of sample E (6.55 g/100g) was significantly ($p<0.05$) high compared to all the other samples. Both phenylalanine and tyrosine can be classified as aromatic amino acids. Phenylalanine is nutritionally indispensable while tyrosine is a metabolic product of the former catabolism. Thus, tyrosine is dependent on sufficient phenylalanine to meet the needs for these two amino acids (WHO/FAO/UNU, 2007). The alanine of wheat-red kidney biscuit ranged from 2.04 to 5.15 g/100g. The value of alanine content increased with an increase in the substitution of red kidney bean flour in the biscuit. Alanine content of sample E (5.15 g/100g) was significantly ($p<0.05$) high compared to all the other samples. The functions of alanine include inhibition of pyruvate kinase and hepatic autophagy, gluconeogenesis, transamination, and glucose–alanine cycle (Duan *et al.,* 2015).

The main non-essential amino acid found in the biscuits samples was glutamic acid, with values ranging from 3.71–8.68 g/100 g. Nutritionally, the percentage ratios of total of the essential amino acid to total amino acid in the red kidney and wheat flour biscuit blends were all above the values considered to be adequate for ideal protein for children and adults (FAO, 2007). These essential amino acids in the composite biscuits may adequately support growth and development in children and adults (Adegbanke *et al.,* 2024). The addition of red kidney bean flour in the biscuit resulted to a biscuit with improved protein quality which can support growth in humans.

Protein efficiency ratio (PER) is one of the measurable protein quality indices which determinates the effectiveness of proteins through the measurement of growth of rats (Arogba and Matanmisi, 2014). The Protein Efficiency Ratio (PER) of the experimental rats ranged from 1.38 to 2.71. The rats fed with 100% wheat biscuit (A1) had the least PER and was significantly ($p>0.05$) different from AA (casein diet), C1 (rat fed diet of 80% wheat and 20% red kidney bean), D1 (rat fed diet of 70% wheat and 30% red kidney bean), and E1 (rat fed diet of 60% wheat and 40% red kidney bean), but, was similar to B1 (rat fed diet of 90% wheat and 10% red kidney bean). The casein diet was not significantly ($p>0.05$) different from B1, C1, D1 and E1. This shows that rats fed these diets (B1, C1, D1 and E1) gained weight as the nitrogen intake increased. The protein efficiency ratio (PER) is used as a good estimate of protein quality (Arogba and Matanmisi, 2014). Protein quality is the ability of a food to support body growth and maintenance. Growth is the increase in size and body weight. The PER result was within the ranges (2.24 to 2.66) obtained by El-Hadidy *et al.* (2020) who worked on biscuit made from quinoa, naked barley and carrot and similar to the result (1.20 to 2.70) reported by Umerah *et al.* (2020) who worked on complementary food from maize, crayfish and carrot. Also similar to the result (2.16 to 2.50) of Okoye *et al.* (2016) who worked on wheat/soybean and bambara groundnut biscuit but slightly lower than the result (2.87 to 3.02) from Kumar *et al.* (2019) who worked on influence of multigrain premix on nutritional, in-vitro and in-vivo protein digestibility of multigrain biscuit. The gradual increase of the PER of the feed diet having higher kidney beans indicates that the diet has a promising essential amino acid content.

Biological value (BV) measures the percent of absorbed protein retained in the body. The biological value of the diets of the experimental rats ranged from 68.27 to 96.29% with the rats fed casein diet (AA) having the highest value and was significantly ($p<0.05$) different form all the other diets while rats fed diet of 100% wheat (A1) had the least BV. The rats fed diet of 70% wheat and 30% red kidney bean (D1) and rats fed 60% wheat and 40% red kidney bean (E1) was not significantly ($p>0.05$) different from each other. While all the other diets were significantly ($p<0.05$) different form each other. The biological value of D1 and E1 were the highest when compared with the positive control diet (AA). The result of this study shows that the utilization of the protein consumed in the diet was highest in D1 and E1. The biological values obtained in this study which was above 80% can support human growth and maintenance with adequate energy intake. This result was similar with the works of Okoye *et al.* (2016) who had BV (84.64 to 92.82%) on wheat/soybean and bambara groundnut biscuit. The BV result was higher than the result (64 to 80%) reported by Umerah *et al.* (2020) who worked on complementary food from maize, crayfish and carrot. Also, it was higher than the result (73.56 to 77.95%) obtained from El-Hadidy *et al.* (2020) who worked on biscuit made from quinoa, naked barley and carrot.

The True Protein Digestibility (TPD) of the diets of the experimental rats ranged from 49.43 to 92.09%. The result of the TPD of all the diets fed to the experimental rats showed that they were significantly ($p<0.05$) different from each other. The positive control, i.e., casein diet (AA) had the highest TPD while it was followed by rats fed diet of 60% wheat and 40% red kidney bean (E1). The lowest in TPD was rats fed diet of 100% wheat. The increase in TPD of feed diet having higher kidney beans indicates that the diet can be digested and absorbed by the body because of the quality of essential amino acids present in the feed. The True Protein Digestibility (TPD) results obtained which was between 49.43-92.09% was similar with the result (70 to 97.64) of Umerah *et al.* (2020) who worked on complementary food from maize, crayfish and carrot. The result (63-83%) from Nosworthy *et al.* (2018) who worked on the effect of processing on the in vitro and in vivo protein quality of beans was slightly different from the result obtained in this study. The difference may be due to the end products in the work, as one was diet formulated with red kidney bean the other was on the processing of the bean.

Net Protein Utilization (NPU) measures percent of dietary protein retained in the body (Adhikari *et al.,* 2022). The Net Protein Utilization (NPU) of the diet of the experimental rats ranged from 65.63 to 94.59%. The casein diet (AA) had the highest NPU and was followed by rats fed diet of 60% wheat and 40% red kidney bean (E1). All the diets were significantly ($p<0.05$) different from each other. The rats fed diet of 100% wheat (A1) had the least NPU. The Net Protein Utilization (NPU) of 100 indicates 100% utilization of dietary nitrogen as protein (Cherian, 2019). The Net Protein Utilization (NPU) of 80% and above recorded in this study shows that the diets has high amount of essential amino acid and could be utilized by the body for growth and maintenance of cells and tissues The high NPU recorded in the rats fed with wheat/red kidney bean diets was due to lower urinary output which lead to increase in retained nitrogen. The result obtained in this study which was between 88-96% was similar to the result of Okoye *et al.* (2016) who worked on wheat/soybean and bambara groundnut biscuit. But the result was higher than the result (62 to 77 %) reported by Umerah *et al.* (2020) who worked on complementary food from maize, crayfish and carrot.

5. Conclusion

Amino acid profile shows that lysine content of samples D and E which are 70:30 and 60:40 are biscuit made from wheat and red kidney bean flours, respectively were higher than all the other samples. Also, sample E (60% wheat flour and 40% red kidney bean flours) had the highest score in the sulphur containing amino acids, methionine and cysteine. When it came to the non-essential amino acids, samples D and E took the lead and was significantly ($p<0.05$) high compared to other samples. It was observed that the higher the red kidney beans flour were in the biscuit the higher the amino acid composition.

The study shows that PER, BV, NPU and TPD scores of rats fed casein diet (positive control) was higher than that of the experimental diets. Generally, the PER, BV, NPU and TPD of the rats fed 70:30 and 60:40 wheat and red kidney bean, respectively compared closely to the casein diet. The incorporation of red kidney beans flour into wheat flour biscuit increased the amino acid profile and improved nutritional quality of the biscuit. Therefore, this research has shown that there is a possibility of the biscuits to support growth and maintenance of the body in both human and animal subjects.

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