



Amino Acid Profile/Score and *In-vitro* Protein Digestibility of Biscuits Produced from Wheat Flour, African Breadfruit Flour and Moringa Seed Flour Blends

V. C. Wabali^{1*}, S. Y. Giami², D. B. Kiin-Kabari² and O. M. Akusu²

¹*Department of Food, Home Science and Nutrition, Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Nigeria.*

²*Department of Food Science and Technology, Faculty of Agriculture, Rivers State University, Nkpolu- Oroworukwo, Port Harcourt, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author VCW performed the study and the statistical analysis, wrote protocol and wrote the first draft of manuscript. Authors SYG, DBKK and OMA supervised and approved the research. Author OMA reviewed and edited the manuscript. All authors approved the manuscript.

Article Information

DOI: 10.9734/AFSJ/2020/v18i130208

Editor(s):

(1) Dr. Uttara Singh, Panjab University, India.

Reviewers:

(1) Ravelly Casarotti Orlandelli, Universidade Estadual de Maringá, Brazil.

(2) Fernando Ramos-Escudero, Universidad San Ignacio de Loyola, Perú.

(3) PuFu Lai, Fujian Academy of Agricultural Sciences, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/60164>

Original Research Article

Received 13 June 2020
Accepted 18 August 2020
Published 14 September 2020

ABSTRACT

The objective of this work was to evaluate the Amino Acid profile/score and In-vitro protein digestibility of composite biscuits produced from blends of Wheat flour (WHF), African breadfruit flour (ABF) and Moringa seed flour (MSF) at the following ratios (Sample A: WHF 100%: ABF 0; MSF 0, B= WHF 77.5%:ABF 20%: MSF 2.5%, C=WHF 75%: ABF 20%: MSF 5.0%, D= WHF 72.5%: ABF 20%: MSF 7.5%. E = WHF 70%: ABF 20%: MSF 10%, F = WHF 90%: ABF 0: MSF 10%, G = WHF 80%: ABF 20%: MSF 0). The most predominant Amino Acid in ABF was glutamic (12.27 g/100 g) followed by Aspartic and lysine, with values of 8.96 g/100 g and 6.55 g/100 g, respectively. Glutamic Acid content of the biscuits ranged from 10.96 g/100 g – 12.96 g/100 g, with sample B giving significantly higher value. Substitution with MSF resulted in decreasing glutamic

*Corresponding author: Email: victor.wabali@uniport.edu.ng, victor_wabali@yahoo.com;

acid content levels in the formulated biscuits, while lysine, phenylalanine and Isoleucine improved with the addition of 10% Moringa seed flour. Amino acid Scores of the biscuits using Hen egg as standard showed that whole egg had a higher amino acid score except glycine (1.04 – 1.25). Percentage In-vitro protein digestibility ranged from 10.64% - 47.33%, showing that addition of moringa seed flour and African breadfruit flour improved digestibility values from 10.64% to 47.33% for sample E with the control sample (wheat flour biscuit) being significantly lower. Substitution with ABF and MSF improved protein digestibility of the produced biscuits. Also, the Amino acid scores of the formulated biscuits were higher than the FAO recommended daily dietary requirements for Amino acids.

Keywords: Protein quality; biscuit formulation; biscuit production; composite biscuits.

1. INTRODUCTION

The low nutritive value of biscuits is an issue of major concern since wheat flour is low in proteins, mineral elements and vitamins. Therefore, the production of biscuits with higher nutritive value is necessary. Searching for a protein source that will supplement wheat flour with high biological value protein has been a subject of many researchers.

Man et.al. [1] produced functional gluten free biscuits from a blend of different non wheat flours such as rice, maize and soy. The gluten free biscuits exhibited good sensory and nutritional qualities. Also, Giami and Barber [2] produced cookies with flour blends of germinated and ungerminated protein concentrates of Fluted pumpkin at 5 – 20% ratios. The cookies showed significantly higher values of protein efficiency ratio, net protein value and protein digestibility. It was shown that cookies supplemented with pumpkin concentrates from germinated seeds at 15- 25% levels were nutritionally comparable to diets based on casein.

Dietary proteins differ in their capacity to satisfy the metabolic demands of the nine indispensable amino acids. Digestibility and the extent to which amino acid pattern matches that of the requirement pattern are critical for the nutritional quality of single proteins and protein mixtures. In the past, protein quality was measured in growth experiments with rats expressed in parameters like Protein efficiency ratio (PER) and (NPU) Net protein utilization [3]. Protein quality evaluation aims to determine the capacity of food protein sources and diets to meet the protein and essential amino acid needs of the individual.

Protein requirements are defined in terms of intakes required to meet metabolic needs for maintenance, in terms of age group and those associated with normal growth for infants and children, pregnancy and lactation [4]. Evaluation

of protein quality by the Protein Digestibility Corrected Amino Acid Score (PDCAAS) method measures the protein metabolic effectiveness as a dietary intake that meets minimum requirement.

Millward et.al, [5] reported that bio-availability or the capacity of the protein to provide metabolically available nitrogen and amino acids to tissues and organs is a critical factor in protein quality determination. Other critical factors that affect the bioavailability of amino acids are the food matrix in which a protein is consumed and changes caused by the presence of anti-nutrients. Ocke, [6] highlighted that protein quality scores or indices of dietary quality express the overall healthiness of the diet. They are usually developed based on specific dietary pattern that is known to be healthy or based on pre-existing dietary guidelines for the general population or for the prevention of a specific dietary disease. Moringa oleifera is a widely grown genera *Moringaceae*, found in most parts of humid tropical Africa, Asia and South America [7]. The seed of the plant exhibit a characteristic bitter taste which is attributed to alkaloids, Saponins and Cyanogenic glycosides. The presence of polyunsaturated fatty acids, high amounts of protein, Sulphur amino acids and the lack of anti-nutritional factors encourage the use of the seeds as animal feed [8].

The African breadfruit seed is one of the under-utilized and under-exploited forest tree crops in Nigeria, known botanically as *Treculia africana*. African breadfruit seeds have been reported to be a cheap source of protein, carbohydrate, fat, vitamins and minerals [9]. Biscuits currently sold in Nigeria have limited amounts of nutrients and the need for a product that has the potential to supply most of the nutrients needed has become important. The objective of this research is to formulate Composite biscuits produced from Wheat flour (WHF), African breadfruit flour(ABF) and Moringa seed flour (MSF) blends and investigate the effects of addition of these flour

on the protein digestibility, amino acid content and amino acid scores of the prepared biscuits.

2. MATERIALS AND METHODS

2.1 Moringa Seeds (*Moringa oleifera*)

Moringa seeds were procured from a Research farm in Nigeria. The seeds were separated according to size, colour and shape. The seeds of irregular colour and shape were discarded in order to have raw materials of uniform physical characteristics. The sorted seeds were dehulled and used for processing into flour.

2.2 African Breadfruit Seeds (*Treculia africana*)

Pods of the African breadfruit were purchased in Obi- Ngwa Abia State, Nigeria and the matured seeds obtained, was washed, dried and sorted to remove extraneous materials.

2.3 Preparation of African Breadfruit Seed Flour

The preparation of the African breadfruit seed flour was carried out in accordance with the method of Agu et al. [10] as shown in Fig. 1. The seeds were sorted to remove extraneous material, washed and rinsed. The seeds were boiled in tap water (1:3 w/v) for 30 mins. The seeds were allowed to cool and dehulling of the husks carried out and the samples dried for a period of 6 hours for moisture removal and for ease of milling operation at a temperature of 55°C in an electric air oven. Particle size reduction was done by milling with a German blender and the sample sieved with 250 µm aperture size. Packing was done with polyethylene material.

2.4 Preparation of Moringa Seed Flour

Moringa seed flour was prepared as described by Ogunsina et al. [11] with some modifications as shown in Fig. 2.

Dried Moringa seeds were sorted to remove extraneous materials and dehulled manually and the husks discarded. The seeds were milled to flour.

2.5 Biscuit Production

Biscuit production was done according to the procedure of Agu and Okoli [12], with changes in the baking time as shown in Fig. 3. The recipe in

Table 1 was used in the formulation of various biscuit samples. Flour blends, sugar, margarine and baking powder were manually mixed in a mixing bowl to achieve uniformity, then one whipped egg and 5 ml of vanilla flavour added and mixed thoroughly again for about 10 mins to obtain the desired dough quality. The batter was then spread out on a baking table with the aid of a roller. Cutting of the biscuits was done with a biscuit cutter and transferred to aluminium baking trays, whose surfaces have been previously lubricated with margarine to prevent burning. The surfaces of the biscuits were glazed with whipped egg and perforated. This was then transferred to a pre-set oven at 180°C and baked for 25 minutes. The baked biscuits were then removed from the oven, allowed to cool before packaging.

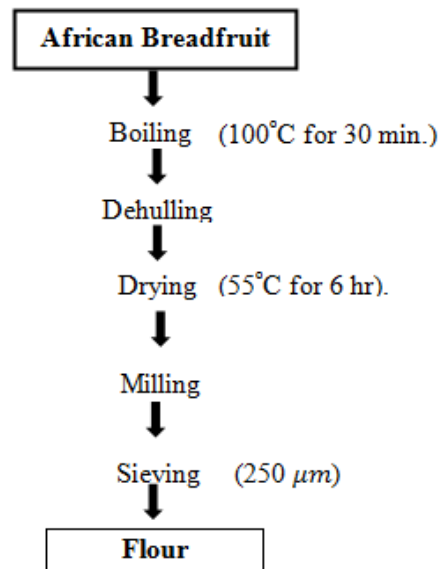


Fig. 1. Preparation of African breadfruit seed flour Agu et al. [10]

2.6 Chemical Analysis

2.6.1 *In-vitro* Protein digestibility determination

In-vitro protein digestibility of the biscuit products was carried out in accordance with the method described by Sauders et al. [13] as reported by Wabali et al. [14].

$$\text{Protein digestibility \%} = \frac{\text{Nitrogen in supernatant} - \text{Nitrogen in blank}}{\text{Nitrogen in sample}} \times 100$$

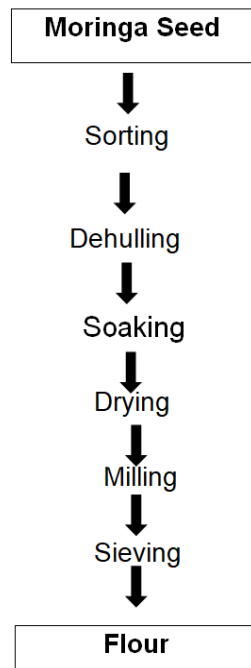


Fig. 2. Preparation of Moringa seed flour
Ogunsina et al. [11]

2.6.2 Determination of amino acid profile

The Amino Acid profile in the known sample was determined using methods described by Benitez [15]. The known sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer (model 120A PTH).

2.6.3 Defatting sample

The sample was defatted using chloroform/methanol mixture of ratio 2:1. Four grams of the sample was put in extraction thimble and extracted for 15 hours in Soxhlet extraction apparatus AOAC, [16].

2.6.4 Nitrogen determination

A small amount (200 mg) of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na₂SO₄), copper sulphate (CuSO₄) and selenium oxide (SeO₂) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 ml) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected. The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V}{W \times C} \times 100$$

Where:

- a. = Titre value of the digested sample
- b. = Titre value of blank sample
- v. = Volume after dilution (100 ml)
- W. = Weight of dried sample (mg)
- C. = Aliquot of the sample used (10 ml)
- 14. = Nitrogen constant in mg.

2.6.5 Determination of tryptophan

The tryptophan in the known sample was hydrolysed with 4.2 M Sodium hydroxide [17]. The known sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

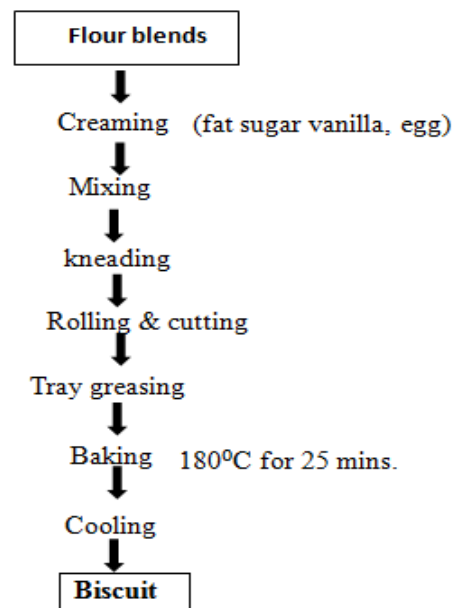


Fig. 3. Flow diagram for baking of biscuits
agu and okoli [12]

Table 1. Recipe for biscuits produced from wheat flour (WHF) African breadfruit Flour (ABF), And Moringa Seed Flour (MSF). (Wabali et al. [14])

Ingredients	Composite biscuit samples						
	A	B	C	D	E	F	G
Wheat Flour (g)	400	310	300	290	280	360	320
Breadfruit Flour (g)	0	80	80	80	80	0	80
M. Seed Flour (g)	0	10	20	30	40	40	0
Sugar (g)	120	120	120	120	120	120	120
Margarine (g)	180	180	180	180	180	180	180
Salt (g)	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Baking Powder (g)	2	2	2	2	2	2	2
Egg (whole)	1	1	1	1	1	1	1
Vanila (ml)	5	5	5	5	5	5	5

Sample A: WHF 100%: ABF 0: MSF 0, B= WHF 77.5%: ABF 20%: MSF 2.5%, C=WHF 75%: ABF 20%: MSF 5.0%, D= WHF 72.5%: ABF 20%: MSF 7.5%. E = WHF 70%: ABF 20%: MSF 10%, F = WHF 90%: ABF 0: MSF 10%, G = WHF 80%: ABF 20%: MSF 0

De-fatting of samples and Nitrogen determination were carried out as previously described.

2.6.6 Amino acid scores FAO/WHO [18]

Amino acid scores were estimated using whole egg as reference protein and the FAO/WHO [18] standard. Estimation of dietary protein quality was done by calculating the amino acid scores using 2 different parameters. The amino acid was calculated using whole hen’s egg amino acid profile using the formula below:

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1g of test protein}}{\text{mg of amino acid in 1g of reference protein}}$$

Also, the amino acid scores were calculated using FAO/WHO reference pattern for adults above 18 years. Determination of aromatic amino acids and sulphur amino acids were calculated from the amino acid profile table.

2.7 Statistical Analysis

Analysis of variance was used to analysed the results obtained and the means were separated using Duncan Multiple Range test at 5% level of probability with the aid of statistical package Genstat 20.0.

3. RESULTS AND DISCUSSION

3.1 Amino Acid Content of African Breadfruit Flour and Biscuit

Results of the Amino acid content of African breadfruit flour are presented in Table 2. The Breadfruit flour contained moderately high amounts of glutamic acid content 12.27 g/100 g, with Aspartic acid having a value of 8.96 g/100 g.

The least amount of amino acid in the flour is Tryptophan with a value of 0.81 /100 g and Cysteine 0.91 g/100 g. The Arginine and the Lysine content of the flour were 6.63 g/100 g and 6.55 g/100 g, and Valine 4.50 g/100 g protein in the African breadfruit flour sample. The results obtained for lysine content of African breadfruit flour were higher than those reported by Cervantes-Palm and Stein [19] for Sesame protein isolate for lysine being 4.78 g/100 g. The values of the other amino acids ranged from 2.56 to 3.85 g/100 g. The presence of these amino acids makes the flour a good source of protein enrichment. The results obtained are in agreement with Nwabueze [20] who reported that glutamic acid and aspartic acid as the most abundant amino acid of the African breadfruits, and recommended that African breadfruit can be used as a substitute for soybean because of its essential amino acid content.

3.2 Amino Acid Contents of Biscuit Samples Produced from Wheat Flour (WHF), African Breadfruit Flour (ABF) and Moringa Seed Flour MSF) Blends

The Amino acid content of biscuit samples produced from wheat flour (WHF), African breadfruit flour (ABF) and Moringa seed flour (MSF) blends is shown in Table 3. The Leucine values of the biscuit formulations ranged from 5.89 g/100 g for the control sample to 6.34 g/100 g for sample F. The results also showed moderately high amounts of glutamic acid content ranging between 10.96 g/100 g and 12.96 g/100 g. Substitution with moringa seed flour resulted in decreasing values of glutamic acid. The results also showed that the glutamic

acid content was higher than all other amino acids in the biscuits formulated with African breadfruit flour.

The result also showed that the following amino acids viz; lysine, phenylalanine, isoleucine had higher values with substitution

of the flour with moringa seed and African breadfruit flour. The value of cystine, Alanine, Tyrosine and Threonine content for sample F was higher than all other formulations of the biscuit showing that 10% moringa seed flour improved these specific amino acids.

Table 2. Amino acid contents of African breadfruit flour (ABF)

Amino Acids	Concentration (g/100 g)
Leucine	5.61 ± 0.01
Lysine	6.55 ± 0.03
Isoleucine	3.70 ± 0.10
Phenylalanine	3.81 ± 0.00
Norleucine	-
Tryptophan	0.81 ± 0.00
Valine	4.50 ± 0.01
Methionine	1.20 ± 0.00
Proline	3.56 ± 0.02
Arginine	6.63 ± 0.03
Tyrosine	3.44 ± 0.00
Histidine	2.56 ± 0.01
Cystine	0.91 ± 0.00
Alanine	3.85 ± 0.01
Glutamic Acid	12.27 ± 0.05
Glycine	3.42 ± 0.01
Threonine	3.85 ± 0.00
Serine	3.84 ± 0.01
Aspartic	8.96 ± 0.03

Values are SD of mean

Table 3. Amino acid contents of biscuit samples produced from wheat flour (WHF), African breadfruit flour (ABF) and Moringa seed flour MSF) blends

Amino acid	Concentration: g/100 g protein						
	A	B	C	D	E	F	G
Leucine	5.89	6.48	5.60	5.31	5.90	6.34	6.19
Lysine	4.13	5.04	4.48	4.14	4.77	5.86	4.53
Isoleucine	3.11	3.70	3.54	3.21	3.67	3.44	3.50
Phenylalanine	3.64	4.43	4.00	3.90	4.26	4.32	4.17
Norleucine	-	-	-	-	-	-	-
Tryptophan	0.88	1.02	0.92	0.84	0.97	0.97	0.95
Valine	4.32	4.79	4.30	4.01	4.50	4.48	4.65
Methionine	1.04	1.39	1.20	1.12	1.23	1.24	1.28
Proline	3.04	3.45	3.15	3.05	3.35	3.38	3.45
Arginine	5.07	6.02	5.33	5.16	5.85	5.94	5.16
Tyrosine	3.04	3.44	3.27	3.10	3.44	3.45	3.10
Histidine	2.16	2.40	2.20	2.17	2.30	2.40	2.40
Cystine	0.95	1.21	0.97	0.91	1.09	1.25	0.97
Alanine	4.12	4.55	4.02	3.83	4.17	4.43	4.29
Glutamic acid	10.96	12.49	11.81	11.28	11.96	11.96	11.96
Glycine	3.13	4.01	3.42	3.30	3.75	3.32	3.61
Threonine	2.44	3.00	2.61	2.39	2.80	3.12	2.89
Serine	3.34	3.73	3.13	3.00	3.40	3.50	3.54
Aspartic acid	7.03	8.00	7.41	7.29	7.57	7.78	7.91

KEY: Sample A: WHF 100%: ABF 0:MSF 0, B= WHF 77.5%:ABF 20%: MSF 2.5%, C=WHF 75%: ABF 20%: MSF 5.0%, D= WHF 72.5%: ABF 20%: MSF 7.5%. E = WHF 70%: ABF 20%: MSF 10%, F = WHF 90%: ABF 0 : MSF 10%, G = WHF 80% : ABF 20%: MSF 0

Table 4. Amino acid scores of biscuit samples produced from wheat flour (WHF), African breadfruit flour (ABF), and Moringa seed flour(MSF) blends

Amino acid	Hens egg	A	B	C	D	E	F	G
Leucine	8.30	0.70	0.78	0.67	0.64	0.71	0.77	0.75
Lysine	6.20	0.66	0.81	0.72	0.67	0.77	0.94	0.73
Isoleucine	5.60	0.55	0.66	0.63	0.57	0.66	0.61	0.63
Phenylalanine	5.10	0.71	0.86	0.71	0.76	0.84	0.85	0.82
Norleucine								
Tryptophan	1.16	0.77	0.88	0.79	0.72	0.84	0.83	0.82
Valine	7.50	0.57	0.64	0.57	0.53	0.60	0.60	0.62
Methionine	3.20	0.32	0.43	0.38	0.35	0.38	0.39	0.40
Proline	3.80	0.80	0.91	0.83	0.80	0.88	0.89	0.91
Arginine	6.10	0.83	0.99	0.87	0.85	0.95	0.97	0.85
Tyrosine	4.00	0.76	0.86	0.82	0.78	0.86	0.86	0.78
Histidine	2.40	0.89	1.00	0.92	0.90	0.96	1.00	1.00
Cystine	1.80	0.52	0.67	0.54	0.51	0.61	0.69	0.54
Alanine	5.40	0.76	0.84	0.74	0.71	0.77	0.82	0.79
Glutamic acid	12.00	0.91	1.04	0.98	0.94	1.00	0.99	1.00
Glycine	3.00	1.04	1.34	1.14	1.10	1.25	1.10	1.20
Threonine	5.10	0.47	0.59	0.51	0.47	0.55	0.65	0.57
Serine	7.90	0.42	0.47	0.40	0.38	0.43	0.44	0.45
Aspartic acid	10.70	0.66	0.75	0.69	0.68	0.71	0.73	0.74

Sample A: WHF 100%: ABF 0; MSF 0, B= WHF 77.5%: ABF 20%: MSF 2.5%, C=WHF 75%: ABF 20%: MSF 5.0%, D= WHF 72.5%: ABF 20%: MSF 7.5%. E = WHF 70%: ABF 20%: MSF 10%, F = WHF 90%: ABF 0: MSF 10%, G = WHF 80%: ABF 20%: MSF 0

Table 5. Amino Acid Score (FAO 2011) of biscuit samples produced from wheat flour (WHF), African breadfruit flour (ABF) and Moringa seed flour(MSF) blends. (mg/g Protein.)

Amino acid	FAO	A	B	C	D	E	F	G
His	15	24	24	24	22	23	24	24
Ile	30	31	37	35	32	36	34	35
Leu	59	59	64	56	53	59	63	62
Lys	45	41	50	45	41	47	58	45
SAA	22	20	26	22	21	23	25	23
AAA	38	75	88	82	78	86	87	82
Thre	23	24	30	26	24	28	32	29
Trp	6.0	8	10	9.0	9.0	10	10	10
Val	39	43	48	43	40	45	45	39

KEY: His.. Histidine, Ile .. Isoleucine, Lue... Leucine, Lys .. Lysine, SAA.. Sulphur amino acid, AAA. Aromatic amino acids, Thre.. Threonine, Trp.. Tryptophan, Val.. Valine, AAA = Phenylalanine + Tyrosine + Tryptophan, SAA = Methionine + Cysteine

Sample A: WHF 100%: ABF 0; MSF 0, B= WHF 77.5%: ABF 20%: MSF 2.5%, C=WHF 75%: ABF 20%: MSF 5.0%, D= WHF 72.5%: ABF 20%: MSF 7.5%. E = WHF 70%: ABF 20%: MSF 10%, F = WHF 90%: ABF 0: MSF 10%, G = WHF 80%: ABF 20%: MSF 0

Results of other amino acids such as methionine, proline, and serine showed that the level of substitution did not have a corresponding significant effect on the amino acid content. The result obtained was in agreement with Okoye et al. [21] who reported an improvement in the amino acid content of biscuits produced from wheat flour, soybean and Bambara groundnut flours.

3.3 Amino Acid Scores of Biscuit Samples Produced from Wheat Flour (WHF), African Breadfruit Flour (ABF) and Moringa Seed Flour (MSF) Blends

The Amino acid scores of biscuit samples produced from wheat flour (WHF), African breadfruit flour (ABF) and Moringa seed flour (MSF) blends using Hen’s egg as standard (Paul et al. [22]) are shown in Table 4. Results

indicated that the amino acid content of the whole egg was higher than the amino acid values of the various biscuit samples formulated except for glycine which had a value 1.04 – 1.25 g/100 g. The glycine content of the biscuit was higher than whole egg from the results obtained. The results showed that substitution of the flour blends with moringa seed flour and African breadfruit flour led to improved glycine content of the biscuits. The most limiting amino acid was methionine, when compared to whole egg with values ranging from 0.32 g/100 g to 0.40 g/100 g for sample G. and Serine with a value of 0.40 g/100 g to 0.47 g/100 g. The results showed that whole egg was a better source of nutritional amino acid than the biscuits formulated with African breadfruit flour and moringa seed flour. The Amino Acid Score of the African breadfruit biscuit evaluated based on the recommendation of FAO [8] for adults above 18 years of age are presented in Table 5. The results obtained from the various formulations of the biscuits indicated that the different amino acid content of the biscuits was higher than FAO recommended values. The results from the formulations showed that there was no limiting amino acid, therefore making the African breadfruit flour a rich source of amino acids.

Table 6. *In vitro* Protein digestibility of biscuit samples produced from wheat flour (WHF), African breadfruit flour (ABF) and Moringa seed flour (MSF) blends

Sample	IVPD (%)
B	30.87 ± 2.36 ^d
C	39.32 ± 0.00 ^c
D	42.80 ± 0.00 ^b
E	47.33 ± 1.77 ^a
F	25.73 ± 0.06 ^e
G	30.35 ± 2.07 ^d

Values are Mean ± SEM of duplicate determination. Means with different superscripts within a column are significantly different (*p* 0.05).

Sample A: WHF 100%: ABF 0; MSF 0, B= WHF 77.5%:ABF 20%: MSF 2.5%, C=WHF 75%: ABF 20%: MSF 5.0%, D= WHF 72.5%: ABF 20%: MSF 7.5%. E = WHF 70%: ABF 20%: MSF 10%, F = WHF 90%: ABF 0: MSF 10%, G = WHF 80%: ABF 20% : MSF 0

Results of the *In vitro* protein digestibility of the biscuits presented in Table 6 showed that the addition of moringa seed flour and African breadfruit flour improved digestibility values from 10.64% to 47.33% for sample E with the control sample being significantly lower than all other samples. However, there was no significant difference between samples B and G with values

30.87% and 30.35% respectively. Product sample E containing 70% wheat flour, 20% African breadfruit flour (ABF) and 10% Moringa seed flour was significantly higher than all other samples. Higher protein digestibility values (69.03%) have been reported for composite biscuits made from African walnut flour and Moringa seed flour blends (Wabali et al. [14], Edem et al. [23], Nwaoguikpe et al. [24]. Van DerPlankin et al. [25] reported that increased protein digestibility values could be as a result of contributory effect of soluble proteins such as albumin which are easily hydrolysed by enzymes.

4. CONCLUSION

Amino acid content of African breadfruit flour showed high amounts of acidic amino acids (AAA) such as glutamic acid and aspartic acid, with tryptophan being the most limiting. The Amino acid scores of the formulated biscuits were higher than the FAO dietary recommended standard [8] showing that substitution of wheat flour with African breadfruit flour and Moringa seed flour led to increased amino acid content in the biscuits. Protein digestibility values improved from 10.64 – 47.33% with increased level of substitution with moringa and African breadfruit flours respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Man S, Adriana P, Muste S. Preparation and quality evaluation of gluten free biscuits. Bulletin UASV Food science and Tech. 2014;71(1):2-10.
- Giami SY, Barber L. Utilization of protein concentrates from un-germinated and germinated fluted pumpkin seeds in cookie formulations. Journal of Science, Food and Agric. 2004;84:1901-1907.
- Gertjan S. Advantages and limitations of PCDAAS as a method of evaluating protein quality. British Journal of Nutrition. 2012;108:333–336.
- FAO. Dietary Protein quality evaluation in human nutrition. WHO/FAO expert consultation report: 92. (DIAAS). FAO, Geneva; 2013.
- Millward DJ, Layman D, Tome D, Schaafsma G. Protein quality assessment: Impact of expanding understanding of

- protein and amino acid needs for optimal health. American Journal of Clinical Nutrition. 2008;87(5):1576–1581.
6. Ocke MC. Evaluation of methodologies for assessing overall dietary quality scores and dietary pattern. Proceedings of British Nutrition society. 2013;72:191-199.
 7. Anwar F, Latif S, Ashrafi M, Gilani H. *Moringa oleifera*: A food plant with multiple medicinal uses. Phyto-therapy Research. 2007;21:17–25.
 8. Ferreira P, Farias D, Olivera J, Carvalho A. *Moringa oleifera*: Bioactive compounds and nutritional potential. Rev. Nutrition. Campinas. 2008;21(4):431–437.
 9. Olapede A, Umeonuah U. Mineral, Vitamin and Anti-nutrient content of African breadfruit seeds processed with alum and trona. Journal of Environment, Science, Toxicology and Food Technology. 2013; 5(5):71-78.
 10. Agu HO, Paul AM, Ayo JA, Folorunsho F. Quality characteristics of biscuits made from wheat and African breadfruit seeds. Nigerian Food Journal. 2007;25(2):12–19.
 11. Ogunsina BS, Radha C, Indirani D. Quality characteristics of bread and cookies enriched with debittered *Moringa* seed flour. International Journal of Food Science and Nutrition. 2011;62(2):185–194.
 12. Agu OH, Okolo AN. Physico-chemical sensory and micro-biological assessment of wheat based biscuit improved with beniseed and unripe plantain. Food Science and Nutrition. 2014;2(5):464–469.
 13. Saunders RM, Connor MA, Booth AN, Bickhoff EN, Kohler GO. Measurement of digestibility of alfalfa protein concentrates by *in-vivo* and *in vitro* methods. Journal of Nutrition. 1973;103:530–535.
 14. Wabali VC, Giami SY, Kiin-Kabari D, Akusu M. Physicochemical, antinutrient and *In-vitro* protein digestibility of biscuits produced from wheat, African walnut and Moringa seed flour blends. Asian Food Science Journal. 2020;14(1):17–26.
 15. Benitez LV. Amino acid and fatty acid profiles in aquaculture nutrition studies. In De Silva SS (edn). Fish nutrition research in Asia. Proceedings of 3rd Asian fish nutrition network. Asia Fish society Publication.1989;4.
 16. AOAC. Association of official analytical chemist. Method of analysis, 18th edition. Washington, D.C; 2006.
 17. Robel EJ. Ion-exchange chromatograph for the determination of tryptophan analytical biochemistry. 1984;18:406–413.
 18. FAO. Protein quality evaluation. International symposium on dietary proteins for human health. Food Nutrition paper. New Zealand; 2011.
 19. Cervantes – Palm SK, Stein HH. Ileal digestibility of amino acids in conventional fermented and enzyme treated Soybean meal and Soy protein isolate. Journal of Animal Science. 2010;88:2674–2683.
 20. Nwabueze TU. Nitrogen solubility index and amino acid composition of African breadfruit blends. Effect of extrusion cooking and process variables. Nigerian Food Journal. 2007;25(1):23–35.
 21. Okoye JO, Ojimekwe P, Ukom AN. Amino acid composition and protein quality of wheat flour biscuits fortified with Soybean and Bambara groundnut flours. Global Journal Sci. Engr. Research. 2016; 3(4):8–19.
 22. Paul AD, Southgate AT, Russel J. First Supplement to McCance and Widdowsons the composition of foods. HMSO. London; 1976.
 23. Edem CA, Dosunmu MI, Bassey F. Determination of proximate composition, ascorbic acid and heavy metal content of African walnut. Pakistan Journal of Nutrition. 2009;8(3):225–226.
 24. Nwaoguikpe RN, Ujowundu CO, Wesley B. Phytochemical and biochemical composition of African walnut. Journal of Pharmaceutical and biomedical Sciences. 2012;20(9):1–5.
 25. Van De Plankin I, Van Remoortee M. Heat induced changes in the susceptibility of Egg white proteins to enzymatic hydrolysis: A kinetic study. Journal of Agric Food chemistry. 2003;51:3819–3823.

© 2020 Wabali et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
 The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/60164>