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Amino Acid Composition of Kilishi - Nigerian (Beef Jerky) Meat

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Abstract. The article reports the amino acid composition of Nigerian beef jerky meat called Kilishi. Kilishi is consumed dry, hence determination was on dry weight basis. Sample was purchased in Ado-Ekiti, Nigeria. Amino acid values were highest for non-essential amino acid in Glu (14.3 $g100g^{-1}$) whereas from essential amino acid it was Lys (8.69 $g100g^{-1}$). Other high value amino acids were (in $g100g^{-1}$): Asp (8.85), Leu (7.68), Arg (6.02), Ile (4.08), Trp (1.02), Cys (1.18) and His (2.40). P-$PER_{1,2,3}$ values were superior at values of 2.52 – 2.70. $EAAI_1$ (soybean standard) was 1.23 and $EAAI_2$ (egg standard) was 94.5 with corresponding BV of 91.3. Lys/Trp was very high at 8.55 and Met/Trp was 2.38. Values of TNEAA was 52.1 $g100g^{-1}$ (57.7%) and TEAA was 38.2 $g100g^{-1}$(42.3%). In the egg score comparison Ser (0.461) was the limiting amino acid (LAA) with protein corrected digestibility value of 0.338; in provisional EAA scoring pattern, LAA was Val (0.882) and corrected version was 0.742; in pre-school children requirement, LAA was Trp (0.927) and corrected value of 0.780. Variation percentage values between the scores/corrected scores were virtually 12.2% per parameter compared. Correlation values between each score standard/corrected score values were significantly different at $r_{=0.01}$ with values of 0.9997 – 0.99999. Estimates of amino acid requirements at ages 10 – 12 years (mg kg^{-1} day^{-1}) showed kilishi to be better than the standards at 74.9% - 453%. Results showed that kiishi is protein-condensed.

Introduction

Kilishi is a version of jerky meat that originated in Hausaland (in Nigeria). It is a derived form of suya, made from deboned cow, sheep or goat meat. Each of the selected muscle is sliced into sheets of one metre or less for easy drying. The dried sheets of meat are then collected and kept for the next process [1]. [Jerky is lean trimmed meat that has been cut into strips and dreid (dehydrated) to prevent spoilage]. Normally, this drying includes the addition of salt to prevent bacteria growth before the meat has finished the dehydrating process [2].

A paste made from peanuts, called *labu*, is diluted with enough water, spices, salt, ground onions, and sometimes sweetners such as honey, to add sweetness. A more natural way to add sweetness is by adding date palm. The dried "sheets'' of meat are then immersed one by one in the *labu* paste to coat them, and then left for hours before roasting to taste [3,4].

After roasting, the final moisture content ranged between 10 – 12%, which decreases during storage at room temperature to 7%. When packaged in hemetically sealed low density plastic pack of 0.038mm thickness, kilishi remains appreciably stable at room temperature for a period of about one year [2].

Other ingredients or treatments to improve the quality of kilishi are: use of suya spice (suya pepper); cloves of garlic; one teaspoon of cloves (kanafuru); piece of ginger; stock cube; dry cayenne pepper seeds; the meat used for kilishi should be free from fat and should be gotten from the reddish part of the beef. Each of these items give kilishi special characteristics such as aroma, e.t.c. [5].

The above gave some information on the preparation, food characteristics and ingredients that improve the nutritional quality of kilishi. However, there is paucity of information on the amino acid composition and the digestibility of kilishi. These are the major concern of this work and in addition the nutritional importance of consuming kilishi will be discussed.

Materials and Methods

Collection of samples

Samples of packaged kilishi were purchased from a supermarket in Ado – Ekiti, Ekiti Sate, Nigeria. The Kilishi samples were actually prepared for sale by Golden Datol Ent., Akure, Ondo State, Nigeria. The kilishi sample was labelled to contain beef, onion, garlic, salt, honey, ginger, maggi, groundnut, pepper.

Sample treatment

The kilishi pack were further oven-dried, allowed to cool, blended and packaged in plastic containers and kept in a cool place pending analysis.

Extraction and analysis

Extraction and instrumental analysis were carried out by following AOAC method [6] and Danka et al. [7].

The dried pulverized sample was made to be free of water by ensuring constant weight for a period of time in the laboratory. The sample of 10.0g was weighed into the 250ml conical flask capacity. The sample was defatted by extracting the fat content of the sample with 30ml of petroleum spirit three times with Soxhlet extractor that was equipped with thimble. The sample was hydrolyzed three times for complete hydrolysis to be achieved for the totality of amino acids recovery.

The pulverized and defatted sample was soaked with 30ml of 1M potassium hydroxide solution and was incubated for 48 hours at 110^0C in hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was neutralized to get pH in the range of 2.5-5.0. The solution was purified by cation-exchange solid-phase extraction. The amino acids in purified solutions were derivatised with ethylchloroformate by the established mechanism:

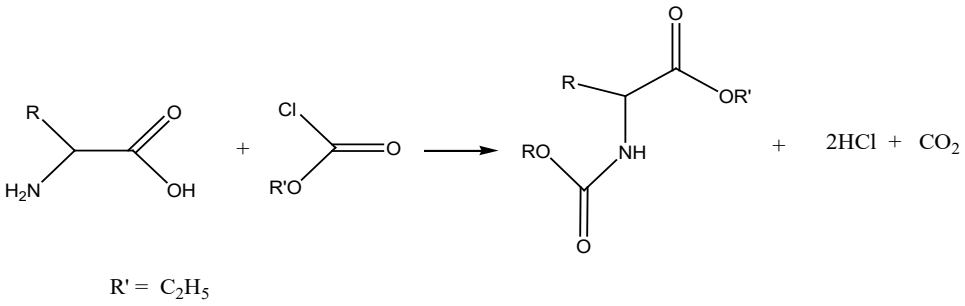

Figure 1. Derivatization process of amino acid

The derivatising reagent was removed by scavenging with nitrogen. The derivatized amino acid was made up to 1ml in a vial for gas chromatography analysis. The gas chromatographic conditions for the amino acids analysis were as follows: GC: HP6890 powered with HP ChemStation rev. AO9.01 [1206] software; injection temperature: split injection; split ratio: 20:1; carrier gas: hydrogen; flow rate: 1.0ml min^{-1}; inlet temperature: 250^0C; column type: EZ; column dimensions: 10m x 0.2mm x 0.25µm; oven programme: initial @ 110^0C, first ramp @ 27^0C min^{-1} to 320^0C ; second, constant for 5mins at 320^0C; detector: PFPD; detector temperature: 320^0C; hydrogen pressure: 20psi; compressed air: 35 psi.

Some calculations were made from the analytical data results.

(i) **Estimation of isoelectric point (*pI*)**: The estimation of isoelectric point (*pI*) for a mixture of amino acids was carried out using the equation of the form [8]:

$$IPm = \sum^{n}_{i=1} I_i P_i X_i \quad (1)$$

where IPm is the isoelectric point of the mixture of amino acids, IiPi is the isoelectric point of the i^{th} amino acid in the mixture and Xi is the mass or mole fraction of the i^{th} amino acid in the mixture.

(ii) **Estimation of predicted protein efficiency ratio (P-PER)**: Computation of protein efficiency ratio (C-PER or P-PER) was done using the equations suggested by Almeyer et al. [9]:

$$P\text{-}PER_1 = -0.468 + 0.454\ (Leu) - 0.105\ (Tyr) \quad (2)$$

$$P\text{-}PER_2 = -0.684 + 0.456\ (Leu) - 0.047\ (Pro) \quad (3)$$

$$P\text{-}PER_3 = -1.816 + 0.435 \times Met + 0.78 \times Leu + 0.211 \times His - 0.944 \times Tyr \quad (4)$$

(iii) **Leucine/isoleucine ratio:** The leucine/isoleucine ratio, their differences and their percentage differences were calculated.

(iv) **Determination of essential amino acid index ($EAAI_2$):** The method of EAAI calculation was due to Oser [10] using the egg protein amino acids as the standard.

(v) **Calculation of biological value (BV):** Computation of biological value (BV) was calculated following the equation of Oser [10]:

$$\text{Biological value} = 1.09\ (EAAI) - 11.73 \quad (5)$$

(vi) **Computation of Lys/Trp and Met/Trp:** The ratios of Lys/Trp (L/T) and Met/Trp (M/T) were computed.

(vii) **Computation of amino acid scores:** The amino acid scores were computed using three different procedures:
- Scores based on amino acid values compared with whole hen's egg amino acid profile [11].
- Scores based on essential amino acid scoring pattern [12].
- Scores based on essential amino acid suggested pattern of requirements for pre-school children [13].

(viii) **Estimation of essential amino acid index ($EAAI_1$):** The essential amino acid index was calculated by using the ratio of test protein to the reference protein for each eight essential amino acids plus histidine in the equation 6 [14]:

$$\text{Essential amino acid index} = \sqrt[9]{\frac{mg\ Lysine\ in\ 1g\ test\ protein}{mg\ Lysine\ in\ 1g\ reference\ protein}} \times \text{etc. for all 8 essential amino acids + His} \quad (6)$$

(ix) **Estimates of amino acid requirements at different ages (mg $kg^{-1}day^{-1}$):** These estimates were based on the essential amino requirements in mg $kg^{-1}day^{-1}$ body weight of 10 - 12 years school boys [13]. The proposed formula for this calculation could be any of these two:
- Essential amino acid x 1000/100 x protein(g $100g^{-1}$) (7)
- Essential amino acid x 10 x appropriate corresponding protein (8)

(x) **Other calculations:** Other determinations such as total amino acid (TAA), total essential amino acid (TEAA), total non-essential amino acid (TNEAA), total acidic amino acid

(TAAA), total basic amino acid (TBAA), total essential aliphatic amino acid (TEAlAA), e.t.c. and their percentages were made. Total sulphur amino acid (TSAA), percentage of cystine in TSAA (% Cys in TSAA) were also calculated. The various amino acid groups into classes I-VII [15] were also calculated.

Determination of Protein Digestibility

The *in-vitro* protein digestibility was determined by the modified method of Akeson and Stahmant [16] and AOAC [6]. The sample containing the exact amount of 100mg of protein was incubated with 1.5mg of pepsin in 15ml of 0.1M hydrochloric acid at a temperature of 38^{o}C for 3 hours. The solution was neutralized with 0.2M sodium hydroxide. Four mg (4 mg) of pancreas in 7.5ml phosphate buffer of pH 8.0 was added with the addition of 1ml of toluene for the prevention of microbial growth and the solution was incubated for another 24 hours at 38^{o}C. The protein content in the solution after 24h of digestion was taken as a measure of the digested product. Following the 24h incubation, the enzyme was inactivated by the addition of 10ml of 10% trichloroacetic acid (TCA) to precipitate undigested protein that was later filtered off. The volume of the filterate was made up to 100ml and centrifuged at 5000 rpm for 30 minutes; the supernatant was collected for protein determination. Blank was digested following the same procedure and employed 1g of each source of enzyme to make protein measurement carried out effectively [17]. The digestibility of the protein was calculated by the equation below:

$$\text{Digestibility} = \frac{Protein\ in\ supernant}{Total\ protein\ of\ the\ sample} \times 100 \quad (9)$$

Determination of Protein Digestibility-Corrected Amino Acid Score

To calculate for protein digestibility-corrected amino acid score for individual foods requires some steps to be taken. These steps are enumerated as follows. Proximate composition must be determined; protein can then be calculated by using a nitrogen-to-protein conversion factor of 6.25. In amino acid profile, protein hydrolysate should be prepared and analysed for amino acid using standard method. Amino acid scores would then be calculated (to give uncorrected amino acid scores). Based on the determined protein digestibility, protein digestibility-corrected amino acid score (PDCAAS) of the test food was then calculated by multiplying the amino acid score x true protein digestibility (or each amino acid score might also be corrected using this similar approach as the case may be). In this report, the score was expressed as a decimal, but it can be expressed in percentage terms [18].

Statistical Evaluation

Data results in Tables 1, 5, 6 and 7 were subjected to statistical analyses of correlation coefficient (r_{xy}), regression coefficient (Rxy), coefficient of determination or variance (r_{xy}^2), the coefficient of alienation (C_A) and index of forecasting efficiency (IFE). Other calculations were grand mean, standard deviation (SD) and coefficient of variation (CV%). The r_{xy} value was converted to critical Table value (r_T) to see if significant differences existed among the various comparisons made in the Tables enumerated above at $r_{=0.01}$ [19].

Results and Discussion

Amino acids encountered in this work:

Lysine (Lys) [PubChem C6H14N202, CID: 5962]; Glutamic acid (Glu) [PubChem C5H9NO4, CID: 33032]; Methionine (Met) [PubChem C5H11NO2S, CID: 6137]; Alanine (Ala) [PubChem C3H7NO2, CID: 5950]; Arginine (Arg) [PubChem C6H14N4O2, CID: 6322]; Valine (Val) [PubChem C5H11NO2, CID: 6287]; Leucine (Leu) [PubChem C6H13N02, CID: 6106]; Aspartic acid (Asp) [PubChem C4H7NO4, CID: 5960]; Threonine (Thr) [PubChem C4H9N03, CID: 6288]; Tryptophan (Trp) [PubChem C11H12N2O2, CID: 6305]; Isoleucine (Ile) [PubChem C6H13NO2, CID: 791]; Phenylalanine (Phe) [PubChem C9H11NO2, CID: 6925665]; Histidine (His)

[PubChem C6H9N3O2, CID: 6274]; Tyrosine (Tyr) [PubChem C9H11NO3, CID: 6057]; Cystine (Cys) [PubChem C6H12N2O4S2, CID: 67678]; Serine (Ser) [PubChem C3H7NO3, CID: 5951]; Glycine (Gly) [PubChem C2H5NO2, CID: 750]; Proline (Pro) [PubChem C5H9NO2, CID: 145742].

PubChem CID

PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Centre for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institute of Health (NIH). Hence we can talk of PubChem Compound ID (CID).

The concentration of amino acids of Kilishi (dry weight) sample in g $100g^{-1}$ is shown in Table 1. In the amino acids (AAs) investigated, glutamic acid (Glu) was the most concentrated with a value of 14.3 g $100g^{-1}$ protein; the next acidic amino acid (AAA), aspartic acid (Asp) was also high at 8.58 g $100g^{-1}$ (ranking as number three in AA concentration). The second most concentrated AA was lysine (Lys) with a value of 8.69 g $100g^{-1}$, this is an essential amino acid (EAA). Other EAA of good concentration were Val, Ile, Leu, Met, His and Trp whereas other nonessential amino acid (NEAA) of good concentration were Gly, Ala, Arg and Cys. The Glu value in this report was lower than the following literature values: heterosexual flesh of *Neopetrolisthes maculatus* (17.7 – 17.8 g $100g^{-1}$ protein) [21]; also the Asp level was lower than the flesh of the heterosexual *N. maculatus* with values of 10.0 – 9.90g $100g^{-1}$ protein [21]. The Glu and Asp levels in the present report were however higher than those observed in the flesh of female West African fresh water crab (*Sadananautes africanus africanus*) with Glu of 130.2 mg g^{-1} protein and Asp of 72.5 mg g^{-1} protein [22]. Studies of Sinclair et al. [23], Schweigert and Payne [24], Mahan and Shields [25] showed EAAs in g $100g^{-1}$: Lys in beef (8.2), lamb (7.5) and pork (7.9), all lower than present report; present Leu value was 7.68 close to their own values of beef (8.5), lamb (7.2) and pork (7.6); Ile was lower at 4.08 compared to their values of beef (5.0), lamb (4.7) and pork (4.8). These EAA values were higher in the literature values in [23, 24, 25] than the present values as shown: (present/literature in g $100g^{-1}$): Val, kilishi/beef (4.41/5.6), kilishi/lamb (4.41/5.1), kilishi/pork (4.41/5.2); Thr, kilishi/beef (3.63/4.2), kilishi/lamb (3.63/4.8), kilishi/pork (3.63/5.2); Met, kilishi/beef (2.42/2.2), kilishi/lamb (2.42/2.4), kilishi/pork (2.42/2.6); Phe, kilishi/beef (3.91/4.1), kilishi/lamb (3.91/3.8), kilishi/pork (3.91/4.3); His, kilishi/beef (2.40/2.8), kilishi/lamb (2.40/2.9), kilishi/pork (2.40/3.1); Trp, kilishi/beef (1.02/1.3), kilishi/lamb (1.02/1.2), kilishi/pork (1.02/1.5). Reports of Beach et al. [26] showed the percentage values of amino acids in beef, lamb and pork: Lys, beef (8.11), lamb (8.68), pork (8.65), all lower than the present Lys (8.69) and His, beef (2.25), lamb (2.37), pork (2.16), all lower than the present His (2.40). In general, Beach et al. [26] observed that muscle tissues of these different classes of animals do not differ widely in their amino acid patterns which implies that the same amino acid composition of muscle proteins is reported throughout the animal kingdom and indicates that, as far as these amino acids are concerned, the protein of one muscle is as good as that of another in supplying amino acids in the diet.

Values of the amino acids shown in Table 1 were subjected to a combined descriptive and inferential statistics as shown in Table 2. The amino acid values were grouped into essential and non-essential amino acids giving a set of nine members each. The mean values were close (4.25 g $100g^{-1}$ EAA – 5.79 g $100g^{-1}$ NEAA) with corresponding standard deviation (SD) values of 2.48 – 3.81 and coefficient of variation (CV%) values of 58.4 – 65.9. Note that the total EAA value was 38.2 g $100g^{-1}$ and the NEAA was 52.1 g $100g^{-1}$ protein. The mean, SD and CV% showed the homogenous nature or otherwise of the EAA and NEAA of the kilishi sample. For the inferential statistics, these values were low: correlation coefficient (r_{xy}) (0.1549), variance (r_{xy}^2) (0.0240) and regression coefficient (Rxy) (0.2381). The r_{xy} was not significantly different since the critical value of 0.798 at $r_{=0.01} > r_c$ of 0.1549. On the other hand the coefficient of alienation (C_A) was high at 0.9879 with correspondingly low index of forecasting efficiency (IFE) of 0.0121. The value of C_A showed that virtually no relationship existed between the kilishi EAA and NEAA since the error of prediction of relationship was high at 98.79% whereas reduction of error of prediction of relationship was just 1.21%.

In Table 3, we depicted the summary of the concentrations of essential, aromatic, non-essential, neutral etc. of the amino acid levels in the sample in g $100g^{-1}$. The total AAs values of 90.3 g $100g^{-1}$ were lower than in the innards of heterosexuals of *N. maculatus* with values of 95.4 – 97.6 g $100g^{-1}$ [27]; value also lower than 96.6 – 97.1 g $100g^{-1}$ in the flesh of the heterosexual *N. maculatus* [21] but higher than the total AAs in the flesh of *S. africanus africanus* (777.0 mg g^{-1} protein) [22]. Columns in Table 3 included AAs class and other quality parameters. Total NEAA (TNEAA) was 52.1 with corresponding percentage value of 57.7. Total EAA (TEAA) was 38.2 (with His) and percentage value of 42.3 whereas values of TEAA without His was 35.8 and the corresponding percentage value was 39.7.

The total aromatic amino acid (TArAA) was 10.4 g $100g^{-1}$ and the TEArAA was 7.32 g $100g^{-1}$ protein; this is within the range of ArAA suggested for ideal protein (68-118 mg g^{-1} protein) [13]; this makes kilishi to be a good source of ArAA and might also be qualified as a supplement to foods of lower ArAA values. The present ArAA value of 10.4 g $100g^{-1}$ protein was higher than in the flesh of *N. maculatus* where values of 7.72 – 9.67 g $100g^{-1}$ protein were reported [21]. The percentage ratio of TEAA to the TAA of the sample was 42.3; this is above 39% considered adequate as ideal food for infants, 26% for children and 11% for adults [13]. The TEAA/TAA in egg is 50% [28]. The total sulphur amino acid (TSAA) had a value of 3.59 g $100g^{-1}$ which is lower than the TSAA recommended for infants (58 mg g^{-1} protein) [13].

The total SAA (TSAA) in the sample were made up of Met + Cys. Whereas the TSAA was 3.59 g $100g^{-1}$ protein, the percentage of Cys/TSAA value was 32.8 which is close to both innards (26.7 – 33.0) and meat of *N. maculatus* at 31.9 – 33.1 [27, 13]. The present %Cys/TSAA (32.8) had close relationships with other animal protein amino acids: 27.3 – 32.8% in *S. africanus africanus* [22], 36.3% in *Macrotermes bellicosus* [29]; 25.6% in *Zonocerus variegatus* [30]; 35.5 in *Archachatina marginata marginata,* 38.8% in *A. archatina* and 21.0% in *Limicolaria* sp. (the last three are land snails consumed in Nigeria [31]. The percentage of Cys in TSAA in the diet of rat chick and pig is 50% [18] but the standard value is unknown to man [13]. All the examples above came from animal sources. It is noted however that vegetable protein (e.g. coconut endosperm) has a percentage Cys/TSAA of 62.8% [32]. Also reported was the high percentage of Cys/TSAA in *Anacardium occidentale* with a value of 50.51% [33]. From these literature values compared with the present result, it is obvious that kilishi AAs behaved like typical animal in their %Cys/TSAA ratios. The presence of cystine and cysteine in the diet reduces the needs for Met and since all the sulphur in the diet is derived from these three AAs, the sulphur content is sometimes used as an approximate assessment of the adequacy of protein [34]. In the present result, the values for Met and Cys were (g $100g^{-1}$ protein) 2.42 and 1.18 respectively.

The predicted protein efficiency ratio (P-PER) was calculated in three forms – P-PER 1, 2 and 3. The *in-vivo* P-PER is of the order of 2.2 [35]. The P-PER values as calculated were: $P\text{-}PER_1 = 2.70$, $P\text{-}PER_2 = 2.62$ and $P\text{-}PER_3 = 2.56$ respectively. Although these three P-PER values were each higher than the *in-vivo* value, all were lower than the following literature values: *N. maculatus* meat where values of $P\text{-}PER_1$ were 3.39 – 3.69 and $P\text{-}PER_2$ were 3.82 – 4.14 [21] and also lower than the report for the innards of *N. maculatus* heterosexuals with values of $P\text{-}PER_1$ (2.83 – 3.01) and $P\text{-}PER_2$ (2.89 – 2.96) [27]. According to Friedman's [36] classification, the PER is poor (< 1.5), moderate (1.5 – 2.0) and superior (> 2.0). On this classification, P-PERs 1, 2, 3 were all in the group of superior category. Other literature P-PER values are: meat of female *S. africanus africanus*, $P\text{-}PER_1$ was 3.1 [22]; in *Callinectes latimanus* (a lagoon crab), $P\text{-}PER_1$ was 1.21 and $P\text{-}PER_2$ was 1.39 [37]. The present P-PER values indicated that kilishi meat might be a more physiologically utilized protein source. In general, it has been discovered that the better the protein, the lower the level in the diet that is required to produce the highest protein efficiency ratio. This emphasizes a clear reflection of the importance of the proper nutritive balance of all amino acids to produce optimum metabolic efficiency. More in Table 3, Leu/Ile ratio was 1.88, Leu – Ile (difference) was 3.59 g $100g^{-1}$, %Leu – Ile/Leu was 46.8. In the meat of *N. maculatus* Leu/Ile ratio had values of 1.60 – 1.63 [21], in meat of *S. africanus africanus*, the ratio was 1.60 [22] and the innards of *N. maculatus* heterosexual the ratios were 1.54 – 1.91 with difference levels of 2.87 – 3.97 g $100g^{-1}$

protein and % (Leu – Ile)/Leu values of 35.1 – 47.7 [27]. From literature, the most ideal Leu/Ile is 2.36 [18]. The value of 1.88 was low to 2.36, hence we might not experience concentration antagonism in the sample when consumed as protein source in food. It has been suggested that an amino acid imbalance from excess Leu might be a factor in the development of pellagra [38]. A high Leu imbalance in the diet impairs the metabolism of Trp and niacin, and is responsible for the niacin deficiency in sorghum eaters [39]. Experiments in dogs have shown that animals fed sorghum proteins with less than 11g $100g^{-1}$ Leu did not suffer from nicotinic acid deficiency [40]. The present Leu value of 7.68 g $100g^{-1}$ protein was much less than 11 g $100g^{-1}$ protein and therefore considered safe and could be beneficially exploited to prevent pellagra in endemic areas [41].

The essential amino acid index (EAAI) calculated were reported in two different forms of $EAAI_1$ and $EAAI_2$. In the $EAAI_1$, the value was 1.23. The $EAAI_1$ under this mode has soybean as its standard for comparison. The value of EAAI in defatted soybean flour is 1.26 [24] and that for whole hen's egg is 1.55. In the amino acid composition of two fancy meats (liver and heart) of African giant pouch rat (*Cricetomys gambianus*), the EAAI ranged from 1.20 – 1.31 [43]. It should be noted that the absence of Trp in EAAI calculation of this mode may bear no significance in the EAAI; for example EAAI without Trp in soy flour remained 1.26 whilst it reduced to 1.54 in the whole hen's egg, i.e. a reduction of 0.01 or 0.645%. For the $EAAI_2$, value was 94.5 with its corresponding biological value (BV) of 91.3 depicting the quality of the kilishi protein. In comparison, some literature values of EAAI and BV are as follows [10]: milk, cow (whole, nonfat, evaporated or dry), EAAI (88) and BV (84, predicted; 90, observed); human, EAAI (87) and BV (83); eggs, chicken (whole, raw or dried, EAAI (100), BV (97, predicted; 96, observed); whites (raw or dried), EAAI (95), BV (92, predicted; 93, observed); yolks (raw or dried, EAAI (93), BV (89, predicted); shellfish (shrimp, including prawns, raw or canned), EAAI (67), BV (61, predicted); also 86.9 – 89.9 (EAAI) and 83.0 – 86.3 (BV) in meat of *N. maculatus* [21] and 88.7 – 89.2 (EAAI) and 85.0 – 85.5 (BV) in innards of *N. maculatus* [27]. These literature results show the quality position of kilishi protein. EAAI is useful as a rapid tool in the evaluation of food formulation for protein quality. The isoelectric point, *pI*, was 5.63 showing the sample to be in the acidic medium of the pH range. The *pI* calculation from amino acids would assist in the quick production of certain protein isolate of an organic product without evaluating the protein solubilty before getting at the *pI*.

Table 3 also contained the Lys/Trp (L/T), Met/Trp (M/T) and Phe/Tyr ratios of the sample. According to Albanese [44], in infants protein requirements, a growth pattern of amino acid requirements was obtained by assigning value of unity to the Trp need. Similar calculation of the amino acid content of mammalian tissue showed that there exists good agreement of growth needs and tissue amino acid patterns. This agreement is said to be good for the L/T and M/T ratios of muscle proteins which constitute approximately 75% of the infant body proteins. The present result had L/T value of 8.55 and M/T value of 2.38. The L/T value was much more than those of innards of *N. maculatus* as 3.00 – 5.01 and meat as 3.31 – 4.27 and also higher than M/T values as: innards, 1.78 – 3.50 and meat, 1.97 – 2.64 [21, 27].

Mammalian tissue patterns have the following values: L/T: muscle (6.3), viscera (5.3), plasma proteins (6.2). M/T: muscle (2.5), viscera (2.0), plasma proteins (1.1) [45]. The available evidence indicates that the utilization of dietary proteins increases as their Lys and Trp approaches that of muscle tissues. In the present study, the kilishi protein L/T value was 8.55 and it is 26.3% greater than the muscle standard of 6.3 L/T, it is also much greater than 5.3 (viscera) and 6.2 (plasma) proteins. Also the M/T value of the present work was 2.38 which is slightly lower than the muscle standard M/T value of 2.5 but higher than 2.0 (viscera), 1.1 (plasma) proteins. The L/T and M/T values were better than our observation for *N. maculatus* innards and meat [21, 27]. The mean minimum Phe requirement estimate in the presence of an excess of Tyr is 9.1 mg kg^{-1} day^{-1}. Hence Tyr can spare 78% of the dietary Phe need. Also the optimal proportions of dietary Phe and Tyr have been shown to be 60 : 40 respectively [46]. The Phe/Tyr in this result was low as shown in Table 3 which did not meet the optimal proportion of dietary Phe and Tyr of 60: 40 respectively.

In Table 4 we have the amino acid groups divided into classes [15]. The concentration trend of the classes follows as shown in g $100g^{-1}$ protien: class I (27.3) > class IV (22.9) > class V (17.1) >

class VI (10.4) > class II (7.27) > class VII (4.23) > class III (3.59). In the innards of *N. maculatus* the trend changed between classes VII and III as shown: class I > class IV > class V > class VI > class II > class III > class VII [27]; this was also the trend in the meat of *N. maculatus* [21]. Further observation would show that the percentage were close to their individual values wih very slight differences; examples were: value (percentage): class I, 27.3 (30.2); class II, 7.27 (8.04); class III, 3.59 (3.98); class IV, 22.9 (25.3); class V, 17.1 (18.9); class VI, 10.4 (11.5); class VII, 4.23 (4.69).

In Table 5, we presented the total amino acid scores based on whole hen's egg amino acids profile and the Protein Digestibility Corrected Amino Acid Score (PDCAAS). Under whole hen's egg score comparison, the following amino acids had scores of 1.00 or > 1.00: Lys, His, Gly, Pro, Ala and Glu. Under PDCAAS comparison, values > 1.00 were recorded only in Lys, Gly and Glu; this is 50% of such values observed in the egg score comparison. Ser was the limiting amino acid (LAA) in both comparisons with values of 0.461 (on egg score) and 0.388 (on PDCAAS comparison). Ser was also limiting with values of 0.511 (male) and 0.487 (female) in the innards of *N. maculatus* and in meat of *N. maculatus* Ser values of 0.513 (male) and 0.516 (female), all on egg score comparisons [21, 27]. To correct the present LAA scores to expected normal level in order to fulfil the day's needs for all the amino acids in kilishi sample, 100/46.1 and (or) 100/38.8; i.e. 2.17 and (or) 2.58 times as much kilishi protein would have to be consumed (eaten) when it serves as the sole protein source in the diet as the case may be. The descriptive statistics in Table 5 showed that the CV% ranged between 11.9 – 12.3. However, in this CV% values, out of 19 values reported, 15/19 or 78.9% had values of 12.2%; 2/19 or 10.5% had values of 12.3; 1/19 was for 12.1 and 11.9 in each case giving a value of 5.26%. Generally speaking, one could reasonably conclude that egg score and PDCAAS comparison had variations of 12.2%. In Table 6, we have EAA scores of kilishi based on FAO/WHO [12] standards. Also there are the PDCAAS scores. For the FAO/WHO [12] scores, these acids had scores > 1.00 : Lys, Met + Cys, Ile, Leu, Phe + Tyr, Trp and total EAA whereas in PDCAAS, only Lys and total EAA had score values > 1.00. Val was the LAA in both comparison scores with values of 0.882 and 0.742 respectively for provisional EAA scores and the PDCAAS. Correction values would therefore be 100/88.2 or 1.13 x protein value and 100/74.2 or 1.35 x protein value for full protein availability for body metabolism. In the CV%, 6/9 or 66.7% had values of 12.2%; 1/9 or 11.1% each for CV% 12.0, 12.1 and 12.3. In Table 7, we have depicted the EAA scores of kilishi based on requirements or pre-school child (2 – 5 y). In the pre-school requirement score comparison only Trp had score < 1.00 and hence LAA with a value of 0.927. Correction value here was 1.08 x protein of kilishi. For PDCAAS, score values > 1.00 were in Lys, Met + Cys, Val, Ile, His and total EAA. Also here Trp was the LAA with a value of 0.780, hence correction was 1.28 x kilishi protein to get balanced protein source. Here, among the CV% values 6/10 (60.0%) were 12.2%; 3/10 (30.0%) were with 12.3 and 1/10 or 10.0% was for 12.1%. Protein Digestibility Corrected Amino Acid Score (PDCAAS) is a method of evaluating the protein quality, with a maximum possible score of 1.0. Most animal meats like beef have a score of approximately 0.9, compared to values of 0.5 – 0.7 for most plant foods [47].

The results of the scores from the egg/PDCAAS, provisional scoring pattern/PDCAAS and pre-school child requirement/PDCAAS from Tables 5, 6, 7 were subjected to statistical analyses as depicted in Table 8. In all the comparisons, all r_{xy} values were high and positively significant. These values were also high: r_{xy}^2, Rxy and IFE. Since all the IFE values were high at 0.9925 – 0.9962, it meant that the quality criteria as depicted in the various scores from the standard score comparisons and the PDCAAS values could be used to ascertain the kilishi quality at virtually similar levels.

In Table 9, we have estimates of amino acid requirements at ages 10 – 12 years in mg kg^{-1} day^{-1} at the body weight of 30 kg. The protein of the kilishi sample had values greater than the estimates in all the amino acids to the tune of between 74.9 – 453%. Among the four principal limiting amino acids of Lys (first), Met + Cys (second), Thr (third) and Trp (fourth), the percentage kilishi protein excess values were: Lys (211%), Met + Cys (185%), Thr (123%) and Trp (448%).

The various amino acids have different types of functions in the human body, phenylalanine, a precursor for neurotransmitters which helps in the production of other amino acids and their functioning. Valine helps in stimulating muscle growth, regeneration and it produces energy.

Threonine is a principal component of structural proteins such as collagen and elastin which are present in skin and connective tissues, helps in fat metabolism and immune function. Tryptophan is a precursor to serotonin, a neurotransmitter that helps in appetite, sleep and mood regulation. Methionine plays a major role in metabolism, detoxification, helps in tissue growth and in the absorption of minerals such as zinc and selenium needed by the body. Leucine helps in regulating blood sugar levels, enhances wound healing and stimulates growth hormones. Isoleucine helps in muscle metabolism, immune function, haemoglobin production and energy regulation. Branched-chain amino acids are Val, Leu and Ile. Lysine helps in protein synthesis, calcium absorption, immune function, energy production, hormone production and in collagen production. Histidine, a neurotransmitter helps maintaining the protective barrier called myelin sheath that surrounds the nerve cells, helps in digestion, immune response, sleep – wake cycles and sexual functions [48].

Conclusions

Kilishi (Nigerian Beef Jerky) meat will serve as a good source of animal protein as well as high quality amino acids. The crude protein value was 64.4g $100g^{-1}$. In amino acid quality parameters, P-PER was in the superior group of value (> 2.0), both EAAI, BV and protein digestibility were high; both Lys/Trp and Met/Trp were high. Scores at standard forms and PDCAAS were highly comparable with slight variation of mostly 12.2%. Estimates of amino acid requirements at ages 10 – 12 years (mg kg^{-1} day^{-1}) in kilishi were all higher than the standards by a range of 74.9 – 453%. Based on the above, kilishi will serve as a healthy snack with a lot of health benefits; it is protein-filled and retains its nutritious value despite being dried.

Table 1. Amino acid profile (g $100g^{-1}$ protein) of Nigerian meat jerky (kilishi)

Amino acid	CID†	Kilishi
Valine (Val)*	6287	4.41
Threonine (Thr)*	6288	3.63
Isoleucine (Ile)*	791	4.08
Leucine (Leu)*	6106	7.68
Lysine (Lys)*	5962	8.69
Methionine (Met)*	6137	2.42
Phenylalanine (Phe)*	6925665	3.91
Histidine (His)*	6274	2.40
Tryptophan (Trp)*	6305	1.02
Glycine (Gly)	750	5.40
Alanine (Ala)	5950	5.70
Serine (Ser)	5951	3.64
Proline (Pro)	145742	4.23
Aspartic acid (Asp)	5960	8.58
Glutamic acid (Glu)	33032	14.3
Arginine (Arg)	6322	6.02
Tyrosine (Tyr)	6057	3.04
Cystine (Cys)	67678	1.18

*Essential amino acid. Determination were done in duplicate and dry weight. †CID = Compound ID.

Table 2. Statistical analysis of the data from Table 1 pertaining to the amino acid profile of essential and non-essential amino acid of kilishi

Statistics	**Essential amino acid**		**Non-essential amino acid**
Total amino acid value	38.2		52.1
Mean	4.25		5.79
Standard deviation (SD)	2.48		3.81
Coefficient of variation (CV%)	58.4		65.9
Correlation coefficient (r_{xy})		0.1549	
Variance (r_{xy}^2)		0.0240	
Regression coefficient (Rxy)		0.2381	
Coefficient of alienation (C_A)		0.9879	
Index of forecasting efficiency (IFE)		0.0121	
Remark		Results not significantly different	

Results not significantly different at n – 2 and $r_{=\ 0.01}$ (critical value = 0.798). (NOTE: n – 2 = 9 – 2 = 7.)

Table 3. Composition in terms of different classes of amino acids in kilishi sample (g $100g^{-1}$ protein)

Parameter	**Kiishi**
Total amino acid (TAA)	90.3
Total non-essential acid (TNEAA)	52.1
% TNEAA	57.7
Total essential amino acid (TEAA)	
With His	38.2
Without His	35.8
%TEAA	
With His	42.3
Without His	39.7
Total aliphatic amino acid (TAIAA) (CLASS I)	27.3
%TAIAA	30.2
Total essential aliphatic amino acid (TEAIAA)	16.2
%TEAIAA	17.9
Total aromatic amino acid (TArAA) (CLASS VI)	10.4
% TArAA	11.5
Total essential aromatic amino acid (TEArAA)	7.32
%TEArAA	8.11
Total acidic amino acid (TAAA) (CLASS IV)	22.9
% TAAA	25.3
Total basic amino acid (TBAA) (CLASS V)	17.1
%TBAA	18.9
Total neutral amino acid (TNAA)	49.3
% TNAA	54.6
Total hydroxyl amino acid (THAA) (CLASS II)	7.27
% THAA	8.04
Cyclic amino acid (Pro) (CAA) (CLASS VII)	4.23
% CAA	4.69
Total sulphur amino acids (TSAA) (CLASS III)	3.59

% TSAA	3.98
% Cys in TSAA	32.8
Leu/Ile ratio	1.88
(Leu-Ile) difference	3.59
% (Leu- Ile)/Leu	46.8
% (Leu- Ile)/total amino acid profile	3.98
$P\text{-}PER_1$,i.e. -0.468+0.454 (Leu)-0.105 (Tyr)	2.70
$P\text{-}PER_2$, i.e. -0.684+0.456 (Leu)- 0.047 (Pro)	2.62
$P\text{-}PER_3$, i.e. -1.816+0.435 x Met + 0.78 x Leu + 0.211 x His – 0.944 x Tyr	2.52
Calculated isoelectric point (*pI*)	5.63
Essential amino acid index: $EAAI_1$	1.23
$EAAI_2$	94.5
Biological value (BV)	91.3
Lys/Trp or L/T	8.55
Met/Trp or M/T	2.38
Phe/Tyr	1.29
Protein digestibility	84.1%

Table 4. Amino acid groups of kilishi

Class	**Value in g 100 g^{-1} protein**	**Percentage value**
I. [with aliphatic side chains (hydrogen and carbon)= Gly, Ala, Val, Leu, Ile]	27.3	30.2
II. [with side chains containing hydroxylic (OH) groups= Ser, Thr]	7.27	8.04
III. [with side chains containing sulphur atoms = Cys, Met]	3.59	3.98
IV. [with side chains containing acidic groups or their amides = Asp, Glu]	22.9	25.3
V. [with side chains containing basic groups = Arg, Lys, His]	17.1	18.9
VI. [containing aromatic rings = His, Phe, Tyr, Trp]	10.4	11.5
VII. [imino acids = Pro]	4.23	4.69

Table 5. Amino acid scores of kilishi based on whole hen's egg amino acid

Amino acid	Egg score	Corrected score	Mean	SD	CV%
Val	0.588	0.495	0.542	0.066	12.2
Thr	0.711	0.598	0.655	0.080	12.2
Ile	0.729	0.613	0.671	0.082	12.2
Leu	0.925	0.778	0.852	0.104	12.2
Lys	1.40	1.18	1.29	0.156	12.1
Met	0.756	0.636	0.696	0.085	12.2
Phe	0.767	0.645	0.706	0.086	12.2
His	1.00	0.841	0.921	0.112	12.2
Trp	0.567	0.477	0.522	0.064	12.2
Gly	1.80	1.52	1.66	0.198	11.9
Ala	1.06	0.892	0.976	0.119	12.2
Ser	0.461	0.388	0.425	0.052	12.2
Pro	1.11	0.934	1.02	0.124	12.2
Asp	0.802	0.674	0.738	0.091	12.3
Glu	1.19	1.00	1.10	0.134	12.2
Arg	0.987	0.830	0.909	0.111	12.2
Tyr	0.760	0.639	0.700	0.086	12.2
Cys	0.656	0.551	0.604	0.074	12.3
Total	0.904	0.760	0.832	0.102	12.2

Table 6. Essential amino acid scores of kilishi based on FAO/WHO [12] standards

Amino acid	FAO/WHO, 1973 based score	Corrected score	Mean	SD	CV%
Lys	1.58	1.33	1.46	0.177	12.1
Thr	0.908	0.763	0.836	0.103	12.3
Met + Cys	1.03	0.866	0.948	0.116	12.2
Val	0.882	0.742	0.812	0.099	12.2
Ile	1.02	0.858	0.939	0.115	12.2
Leu	1.10	0.925	1.01	0.124	12.2
Phe + Tyr	1.16	0.976	1.07	0.130	12.2
Trp	1.02	0.858	0.939	0.115	12.2
Total	1.28	1.08	1.18	0.141	12.0

Table 7. Essential amino acid scores of based on requirements of pre-school child (2-5years) standards [13]

Amino acid	Pre-school child requirement based score	Corrected score	Mean	SD	CV%
Lys	1.50	1.26	1.38	0.170	12.3
Thr	1.07	0.900	0.985	0.120	12.2
Met + Cys	1.44	1.21	1.33	0.163	12.3
Val	1.26	1.06	1.16	0.141	12.2
Ile	1.46	1.23	1.35	0.163	12.1
Leu	1.16	0.976	1.07	0.130	12.2
Phe + Tyr	1.10	0.925	1.01	0.124	12.2
Trp	0.927	0.780	0.854	0.104	12.2
His	1.26	1.06	1.16	0.141	12.2
Total	1.43	1.20	1.32	0.163	12.3

Table 8. Summary of the statistical analyses of the scores reported in Tables 5, 6 and 7

Statistics	Egg scores versus corrected scores	Provisional scoring pattern versus corrected scores	Pre-school child requirement versus corrected scores
r_{xy}	0.99999	0.99999	0.99997
r_{xy}^2	0.99998	0.99997	0.99994
Rxy	0.8447	0.8444	0.8388
$Mean_1$	0.9038	1.11	1.26
SD_1	0.3184	0.2144	0.1948
$CV\%_1$	35.2	19.3	15.5
$Mean_2$	0.7606	0.9331	1.06
SD_2	0.2689	0.1811	0.1634
$CV\%_2$	35.4	19.4	15.4
C_A	0.0038	0.0054	0.0075
IFE	0.9962	0.9946	0.9925
Remark	Significantly different	Significantly different	Significantly different

Egg score is significantly different at n-2 [n-2 = 19 – 2 = 17 (df)] and $r_{=0.01}$ (critical value = 0.575); provisional score is significantly different at n-2 [n-2 = 9 – 2 = 7 (df)] and $r_{=0.01}$ (critical value = 0.798); pre-school child score is significantly different at n-2 [n-2 = 10 – 2 = 8 (df)] and $r_{=0.01}$ (critical value = 0.765).

Table 9. Estimates of amino acid requirements at ages 10-12 years (mg kg^{-1} day^{-1})

Amino acid	School boys (10-12y) = R	School boys 30kg x R = S	Kilishi equivalent = T	Difference (S-T)	Percentage difference (S-T %)
Ile	30	900	2628	-1728	-192
Leu	45	1350	4946	-3596	-266
Lys	60	1800	5596	-3796	-211
Met+Cys	27	810	2312	-1502	-185
Phe+Tyr	27	810	4476	-3666	-453
Thr	35	1050	2338	-1288	-123
Trp	4	120	657	-537	-448
Val	33	990	2840	-1850	-187
Total EAAs	261	7830	31215	-23385	-74.9

- T > S; T was calculated as: specific amino acid x 10 x appropriate corresponding protein value.

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