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Amino Acid Characteristics of Nigerian Local Cheese ('Wara')

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ABSTRACT

This article reports the amino acid composition of the Nigerian local cheese called 'wara'. 'Wara' is made by boiling cow milk with some added coagulant to cuddle the milk protein resulting in coagulated milk protein and whey. 'Wara' used to be an excellent source of nutrients such as proteins, fats, minerals and vitamins. Samples were purchased in Ado-Ekiti, Nigeria. Amino acid values were high (g/100g crude protein) in Leu, Asp, Glu, Pro, Phe, Arg with total value of 97.7. The quality parameters of the amino acids were: TEAA (42.6g/100g and 43.6%) whereas TNEAA (55.1g/100g and 56.4%); TArAA (12.8g/100g and 13.1%); TBAA (14.2g/100g and 14.5%); TSAA (3.10g/100g and 3.17%); %Cys in TSAA (51.4); Leu/Ile ratio (1.74); P-PER$_1$ (2.65); P-PER$_2$ (2.48); P-PER$_3$ (2.41); EAAI$_1$ (soybean standard) (1.29) and EAAI$_2$ (egg standard) (99.9); BV (97.2) and Lys/Trp ratio (3.62). The statistical analysis of TEAA/TNEAA at r=0.01 was not significantly different. On the amino acid scores, Met was limiting (0.459) at egg comparison, Lys was limiting at both FAO/WHO [24] and preschool EAA requirements with respective values of 0.966 and 0.97. Estimates of essential amino acid requirements at ages 10-12 years (mg/kg/day) showed the 'wara' sample to be better than the standard by 3.72-330% with Lys (3.72%) being least better and Trp (330%) being most. The results showed that 'wara' is protein-condensed which can be eaten as raw cheese, flavoured snack, sandwich filling or fried cake.

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Introduction

One of the numerous products from the processing of milk is cheese [1]. In milk producing countries, large fraction of the milk produced is used in cheese making [2]. Cheese is often used as a form of preserving essential nutrients in milk and is an excellent source of nutrients such as proteins, fats, minerals and vitamins. About one-third of the total volume of milk produced in African countries is used in the production of cheese [3]. Widespread traditional manufacture of soft cheese locally called 'wara' occurs in Nigeria. 'Wara' is eaten in various forms such as raw cheese, flavoured snack, sandwich filling or fried cake [4]. Cheese or 'wara' and 'tofu' are produced at household level using coagulants such as CaCl2.2H20, alum and steepwater (from pap produced from maize) [5]. Coagulant used in cheese making has a dual role. The primary function is to coagulate milk to produce cheese curd that is, converting liquid milk to a gel form. This conversion is catalysed by different proteases [6]. In addition, a small proportion of the coagulant is carried over into the cheese. This residual coagulant remains proteolytically active in most aged cheeses and plays an important role in the development of texture and flavour [7]. The grading of milk is according to the concentration of total protein and milk fat. Milk suitable for cheese making must be milk with good clotting properties and high cheese yield [8-9].

Due to lack of industrial manufacture of cheese in Nigeria, the nutritional benefits have not been fully derived. Nigerian cheese called 'wara', is usually manufactured from a coagulant obtained from the juice of *Calotropis procera* (Sodom Apple leaves: *bomubomu* in the Yoruba language). Other sources of coagulants suitable for the production of 'wara' include steep water, $CaCl_2.2H_2O$ and alum.

As sometimes portrayed, 'wara' is not cheese. It is milk curds achieved by adding a coagulant to fresh milk whereas cheese is achieved through a process of ageing pressed milk curd. ['Wara' is not a product of fermentation.] [10]. 'Wara' is made by boiling milk with some added coagulant to curdle the milk protein. The results- coagulated milk protein and whey (the liquid remaining after milk has been curdled and strained). This milk protein is then skimmed off from the whey and sold as '*wara*' [10].

The use of vegetable extracts as milk coagulants in soft cheese processing has been known traditionally in some parts of West Africa like Nigeria and Benin Republic [11]. Milk coagulants of plant origin have over-ridden the use of animal rennet. Type of coagulant is one of the factors that determines the quality and yield of cheese. Plant sources of coagulants include sunflower [11], pineapple [12], several plant preparations of *Cynara cardunculus* [13] and $CaCl_2$ [5]. *Calotropis procera* Linn is small, erect and compact shrub, which is used in several traditional medicines to cure various diseases. *C. procera* Linn is an erect, tall, large, much

branched and perennial shrubs or small trees that grow to a height of 5.4m, with milky latex throughout. Different parts of the plant have been reported to possess a number of biological activities such as proteolytic [14], etc. The latex is used as an abortifacient [15], spasmogenic and carminative properties [16], etc. The latex contains the proteolytic enzymes. This article reports on the amino acid characteristics of 'wara' – The Nigerian Local Cheese.

Materials and Method

Collection of Samples

Samples of 'wara' were purchased from the main market of Ado-Ekiti, Ekiti State in the southern part of Nigeria. Purchase was made from the Bororo women who hawk 'wara' in Ado Ekiti. The 'wara' is always in measured sizes; five of such sizes were purchased for analysis.

Sample Treatment

The samples were cut into smaller bits and spread on aluminium tray. The tray was put in an oven and the sample was oven-dried till constant weight was achieved. The sample was then homogenized, sieved using 200mm mesh size and kept in plastic bottles in the refrigerator (2.8°C) pending analysis.

Crude Protein Determination

Crude protein (N x 6.25) of the sample was evaluated by micro-Kjeldahl method [17].

Extraction And Analysis

Extraction and instrumental analysis were carried out following AOAC method and Danka et al. [17,18]. The dried pulverized sample was made to be free of moisture by ensuring constant weight for a period of time in the laboratory. The sample of 10.0g was weighed into 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 hydrolysed 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 48hours at 110°C in hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was neutralised 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:

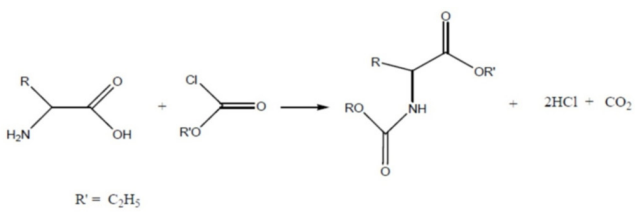

Figure1: Derivatisation process of amino acid

The derivatisation reagent was removed by scavenging with nitrogen. The derivatised 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. A09.01[1206] software; injection temperature: split injection; split ratio: 20:1; carrier gas: hydrogen; flow rate: 1.0ml/min; inlet temperature: 250°C; column type: EZ; column dimensions: 10m x 0.2mm x 0.25µm; oven programme: initial @ 110°C, first ramp @ 27°C/min to 320°C; detector: PFPD; detector temperature: 320°C; hydrogen pressure: 200 psi; compressed air: 35 psi.

Calculations 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 determined using the equation of the form [19]:

$$IPm = \sum_{i=1}^{n} IiPiXi \quad (1)$$

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 Alsmeyer et al. [20]:

P-PER1 = -0.468 + 0.454 (Leu) – 0.105 (Tyr) (2)

P-PER2= -0.684 + 0.456 (Leu) – 0.047 (Pro) (3)

P-PER3= -1.816 + 0.435 x Met + 0.78 x Leu + 0.211 x His – 0.944 x Tyr (4)

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

(iv) Determination 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 5 [21]:

$$\text{Essential amino acid index} = \sqrt[9]{\frac{mg\ Lysine\ in\ 1g\ test\ protein}{mg\ Lysine\ in\ 1g\ reference\ protein}}$$

x etc for all essential amino acids + His (5)

(v) Estimation of essential amino acid index ($EAAI_2$): The method of EAAI calculation was due to Oser [22] using the egg protein amino acids as the standard.

(vi) Calculation of biological value (BV): Computation of biological value (BV) was calculated following the equation of Oser[22]:

Biological value = 1.09(EAAI) – 11.73 (6)

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

(viii) Computation of amino acid scores: The amino acid scores were computed using three different methods;

- Scores based on amino acid values compared with whole hen's egg amino acid profile [23].
- Scores based on essential amino acid scoring pattern [24].
- Scores based on essential amino acid suggested pattern of requirements for pre-school children [25].

(ix) Estimates of amino acid requirements at different ages (mg/kg/day): These estimates were based on the essential amino acid requirements in mg/kg/day body weight of 10-12years school boys [25].

The proposed formula for this calculation could be any of the followings:

- Essential amino acid x 1000/100 x protein (g/100g) (7)
- Essential amino acid x 10 x appropriate corresponding protein (8)

Other calculations: Other calculations made from the analytical results were 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 (TEAIAA), etc. and their percentages. 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 [26] were also determined.

Statistical Evaluation

The amino acids were divided into their groups under essential amino acids and non-essential amino acids groups. This created two groups of nine amino acids per group. These two groups were then subjected to statistical analysis of correlation coefficient (rxy), regression coefficient (R_{xy}), coefficient of determination or variance (r_{xy}^2), the coefficient of alienation (C_A) and index of forecasting efficiency (IFE). Other calculations were mean, standard deviation (SD) and coefficient of variation (CV%). The r_{xy} value was converted to critical Table value (rT) to see if significant differences existed between the two comparisons made in the sample at r = 0.01 [27].

Results

The amino acids (AA) profile of the 'wara' sample had been depicted in Fig. 2. For easy and alternate identification, the compound ID numbers were indicated as appropriate. Amino acid values were reported in g/100g crude protein (cp). The two most concentrated amino acids were Glu (17.3g/100gcp) and Asp (10.1g/100gcp) with corresponding percentage concentration values of 17.7% and 10.3% respectively. Both Glu and Asp were the only acidic amino acids in the sample. The most concentrated essential amino acid (EAA) was Leu at 7.57g/100gcp and 7.75%. Close to this value were Arg (6.03g/100gcp; 6.17%) and Pro (6.10g/100gcp; 6.24%), both are non-essential amino acids (NEAA). Appreciable levels of the amino acids were observed in Lys (5.59g/100gcp; 5.72%), Phe (5.62g/100gcp; 5.76%) and Ser (5.99g/100gcp; 6.14%); both Lys and Phe being essential amino acids. Amino acids with relatively low levels were Met(1.47g/100gcp; 1.50%), Trp (1.55g/100gcp; 1.58%) and Cys (1.63g/100gcp; 1.67%). Both Met and Trp are essential amino acids whereas Cys is sparing partner of Met. The percentage levels of all the amino acids had low differences from the main result values of the amino acids. Such values ranged between 0.03-0.40 with the following percentage difference distributions: 0.03(one AA=5.56%), in this group of one AA/5.56% were: 0.11,0.13, 0.15, 0.18, 0.2 and 0.4 (that is 5.56 x 7= 38.92%; 0.12 (two AA=11.1%), in this group of two AA/11.1% were: 0.07, 0.03 and 0.1 (that is 11.1 x 4 = 44.4%, this came from eight(8) amino acids); 0.14 (three AA=16.7%), this group contained the 0.14 group of three amino acids. Hence, the explanation goes as: seven amino acids (7⁄18) were in the group of 38.9%, eight amino acids (8⁄18) were in the group of 44.4% and three amino acids (3⁄18) were in the group of 16.7%. The total amino acid values was high at 97.7g/100g cp.

The result in Fig. 2 was statistically evaluated as the result was grouped into two subgroups of essential amino acids and non-essential amino acids, each group contained the values of nine amino acids. Calculated for were both descriptive and inferential statistics. For descriptive statistics, we calculated for the mean, standard deviation (SD) and coefficient of variation (CV%). For inferential statistics, the followings were determined: correlation coefficient (r_{xy}), coefficient of determination (r_{xy}^2), coefficient of regression (R_{xy}), coefficient of alienation (C_A) and index of forecasting efficiency (IFE). The calculated rxy was compared to the Table r_{xy} (rT) at r =0.01 to find out if significant relationship existed between the essential and nonessential amino acid values. Results showed the total EAA as 38.0g/100gcp and NEAA as 59.8g/100gcp with corresponding values of mean and CV% as 4.22±2.03g/100gcp, 48.0% and 6.64±4.63g/100gcp, 69.7% respectively. The EAA was much lower than the NEAA acid values and the ratio of EAA to NEAA was 1.00:1.57. Both the man, SD and CV% in EAA were all lower in EAA than in the NEAA. The spread of the values as shown in the SD and CV% in the EAA showed that values were much closer to each other unlike in the NEAA where values were much scattered as shown in higher levels of SD and CV%. In the inferential statistics, all these values were low: r_{xy}, r_{xy}^2, R_{xy} and IFE but value was high in C_A. The r_{xy} value was negative and the R_{xy} also exhibited a negative relationship. The critical Table value of r =0.01 was 0.798 at (df) n-2= 9-2 = 7. Since $r_c = -0.1259 < r_T = 0.798$, then significant difference did not exist in the EAA and NEAA of the 'wara' under discussion. The C_A was high at 0.9920 (99.2%) with a corresponding low value of IFE at 0.0080 (0.8%). C_A is the reverse of IFE or vice versa, the higher the C_A, the lower the IFE; both when added together would either give 100 % (percentage basis) or 1.00 (on fraction basis). This meant that C_A + IFE = 1.00 or 100%. Both C_A and IFE are good in predicting the relationship in two compared set of data. The other action for C_A is that it can be used to predict the relationship or non-relationship of two compared entities, it can also be used to predict the error of prediction. When C_A > IFE, prediction of relationship would be difficult or impossible but it is the reverse when C_A< IFE. Also the C_A value is the error of prediction of relationship. In this result, $C_{A(99.2\%)} >>> IFE_{(0.80\%)}$ showed that the error of prediction was very high at 99.2% thereby making the efficiency of prediction of relationship between EAA and NEAA in 'wara' virtually impossible since the reduction in error of prediction was just 0.80% (IFE). All these were shown in the Table 1.

The quality parameters of the amino acids of the 'wara' studied were depicted in Table 2. In Table 2, the EAA values included the sparing partners of Met (which is Cys) and Phe(which is Tyr) giving a total of 43.6g/100gcp and 43.6% and corresponding NEAA value of 55.1g/100gcp and 56.4%. These values were at variance with the EAA and NEAA values in Table 2 where Cys and Tyr were counted with NEAA to get nine amino acids for EAA and NEAA respectively so that we could calculate for the rxy which must be one/one mapping and in this case 9/9. The total essential aliphatic AA (TEAIAA) was 16.7g/100gcp and 17.1%; total essential aromatic AA (TEArAA) was 9.75g/100gcp and 9.98%; total basic amino acid (TBAA) was 14.2g/100gcp and 14.5%; total sulphur AA (TSAA) was 3.10g/100gcp and 3.17% with 51.4% being the value for %Cys in TSAA. The Leu/Ile ratio was low and nutritionally favourable at 1.74. All the P-PER values were good but decreased from P-PER1 down to P-PER3 as: $P\text{-}PER_1$ (2.65), $P\text{-}PER_2$ (2.48) and $P\text{-}PER_3$ (2.41). The calculated isoelectric point (pI) showed the 'wara' to be in the acid medium (5.56) of the pH scale. The essential amino acid index (EAAI) was high at 1.29 on the soybean (1.26) scale and close to that of egg 99.9 on the egg (100) scale. The biological value was high at 97.2 which showed possible high level of bioavailability. Both Lys/Trp (L/T) and Met/Trp (M/T) values were of moderate concentration. The amino acid groups of 'wara' were shown in Table 3 but the details were further and better shown in Fig. 3. We had classes I to VII, each class depicting the total amino acids of each group. Each class total amino acid values and their corresponding percentage values were shown. The differences in the main value and the percentage value were both low and close as the percentage and main value differences ranged from 0.07 to 0.14. In the percentage differences distribution, we had: 0.07 (only one AA in this group = 14.3%, i.e. 1⁄7 x 100), 0.3(three AAs were in this group = 42.9%, i.e. 3⁄7 x 100), 0.6 (two AAs were in this group = 28.6%, i.e. 2⁄7 x 100) and 0.14 (only one AA in this group = 14.3%, i.e. 1⁄7 x 100); meaning that (1⁄7 x 2 + 3⁄7+ 2⁄7) x 100 gave the percentage/ main value difference distribution in the 'wara' class amino acids. In the class values, class IV, that is total acidic amino acid (TAAA)

of Asp and Glu had the highest concentration of 27.4g/100g cp and 28.0% concentration and the lowest was from class III which was the TSAA from Met + Cys with value of 3.10g/100g cp at 3.17% concentration.

In Fig. 4, we depicted the amino acid scores based on whole egg standard. Amino acids whose scores were greater than 1.00 were : Phe (1.10), His(1.08), Gly (1.62), Pro (1.61) and Glu(1.44); other AAs of 0.9 and above scores were: Leu(0.912), Lys (0.902), Asp (0.944), Arg (0.989), Cys (0.906) and total AA (0.978). The limiting amino acid here was Met with a value of 0.459. Met is the second limiting amino acid after Lys but before Thr and Trp. This meant that Met would be the AA that would need correction to balance the AA profile of 'wara' under this comparison.

In Fig. 5, the essential amino acid scores of 'wara' based on FAO/WHO [24] had been depicted. All the EAA scores with the exception of Lys score had values geater than 1.00 but Lys had a score of 0.966. This made Lys to be automatically the limiting amino acid (LAA) in 'wara' under this standard. Lys is the first limiting amino acid (LAA) in 'wara'; hence Lys would need correction to make 'wara' a complete protein under this standard. As we have in Fig. 5, similar scenario existed in Fig. 6 where we have essential amino acid scores based on requirements of pre-school child (2-5years) standards [25]. All the essential amino acids (including His) with the exception of Lys had scores values greater than 1.00. Lys had a score of 0.97 in Table 7 but value of 0.966 in Table 6 showing a high proximity between the Table values. Since Lys was limiting under this standard, it would need correction in order to obtain a complete protein.

In Fig. 7, estimates of amino acid requirements at ages 10-12years in mg/kg/day were shown as obtained from 'wara'. Column 3 showed the estimates for the school boys as obtained from standards whereas 'wara' equivalent was depicted in column 4. The differences between columns 3 and 4 and the percentage differences were shown in columns 5 and 6 respectively. In column 6, percentage differences ranged between 3.72% – 330% whereas largest percentage difference came from Trp (330%), the least came from Lys (3.72%). The low percentage difference and the very high percentage of other EAAs reflected the observations in Figs. 5 and 6. Since the estimates were all better in 'wara' than in the standard, the results showed that 'wara' could be good for complementation of lower forms of diets with low levels of EAAs.

Discussion

Amino Acids Encountered in This Work

Lysine(Lys) [PubChem C6H14N202, CID:5962]; Glutamic acid(Glu) [PubChem C5H9N04, CID:33032]; Methionine (Met) [PubChem C5H11N02S, CID:6137]; Alanine(Ala) [PubChem C3H7N02, CID:5950]; Arginine(Arg) [PubChem C6H14N402, CID:6322]; Valine (Val) [PubChem C5H11N02, CID:6287]; Leucine(Leu) [PubChem C6H13N02, CID:6106]; Aspartic acid(Asp) [PubChem C4H7N04, CID:5960]; Threonine(Thr) [PubChem C4H9N03, CID:6288]; Tryptophan(Trp) [PubChem C11H12N202, CID:6305]; Isoleucine(Ile) [PubChem C6H13N02, CID:791]; Phenylalanine(Phe) [PubChem C9H11N02, CID:6925665]; Histidine(His) [PubChem C6H9N302, CID:6274]; Tyrosine(Tyr) [PubChem C9H11N03, CID:6057]; Cystine(Cys) [PubChem C6H12N204S2, CID:67678]; Serine(Ser) [PubChem C3H7N03, CID:5951]; Glycine(Gly) [PubChem C2H5N02, CID:750]; Proline(Pro) [PubChem C5H9N02, 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) [28].

The acidic amino acid (Glu and Asp) were the highest concentrated AA in the sample. In Kilishi (Nigerian Beef Jerky), it's amino acid had Glu as the most concentrated amino acid being similar in 'wara'; although 'wara' Glu was higher in value than in Kilishi (17.3/14.3g/100g cp) [29]. Asp was second concentrated in 'wara' but it was third concentrated in Kilishi. The 'wara' Glu (17.3g/100g cp) was comparable to the heterosexual flesh of Neopetrolisthes maculatus (17.7-17.8g/100g cp) [30]; the 'wara' Asp (10.1g/100g cp) was higher than the flesh of the heterosexual N. masculatus with values of 10.0-9.90g/100g cp [30]. Furthermore, Glu and Asp levels in 'wara' were also higher than those in the flesh of female West African fresh water crab (Sudananautes africanus africanus) with Glu of 130.2mg/g cp and Asp of 72.5mg/g cp [31]; Asp in Kilishi was 8.58g/100g cp (being lower than 10.1g/100g cp in 'wara'). Report on Kilishi showed that it's amino acids profile showed higher levels in Leu, Lys, Met, Gly, Ala and Tyr[29] than such amino acids in 'wara' but other AA in Kilishi were lower than in 'wara'. Studies of Sinclair et al [32], Schweigert and Payne [33], Mahan and Shields [34] showed EAAs in g/100g cp: Lys in beef (8.2), lamb (7.5) and pork (7.9), all being higher than in 'wara' (5.59g/100g cp); present Leu value of 7.57g/100g cp was lower than 7.68 in Kilishi [29], 8.5 in beef [32], 7.6 in pork [34] but higher than 7.2 in lamb [33]; Ile (4.34g/100gcp) was lower to beef (5.0), lamb (4.7) and pork (4.8) but higher than 4.08 in Kilishi. These other EAAs had the following comparisons as compared to literature values between 'wara' and the literature sources from references 29, 32, 33, 34 (present/literature in g/100gcp): Val, 'wara'/ Kilishi (4.82/4.41), 'wara'/beef (4.82/5.6), 'wara'/lamb (4.82/5.1), 'wara'/pork (4.82/5.2), Thr, 'wara'/Kilishi (4.41/3.63), 'wara'/beef (4.41/4.2), 'wara'/lamb (4.41/4.8), 'wara'/pork (4.41/5.2), Met, 'wara'/Kilishi (1.47/2.42), 'wara'/beef (1.47/2.2), 'wara'/lamb (1.47/2.4), 'wara'/pork (1.47/2.6), Phe, 'wara'/ Kilishi (5.62/3.91), 'wara'/beef (5.62/4.1), 'wara'/lamb (5.62/3.8) 'wara'/pork (5.62/4.3), His, 'wara'/Kilishi(2.58/2.40), 'wara'/beef (2.58/2.8), 'wara'/lamb (2.58/2.9), 'wara'/pork (2.58/3.1), Trp, 'wara/Kilishi (1.55/1.02), 'wara'/beef (1.55/1.3), 'wara'/lamb (1.55/1.2), 'wara'/pork (1.55/1.5). Reports of Beach et al [35] showed the percentage values of AAs in beef, lamb and pork as: Lys, beef (8.11), lamb (8.68), pork (8.65), which were all higher than 5.59 in 'wara' and His, beef (2.25), lamb (2.37), pork (2.16) all lower than the present His (2.58). Lys was 8.69 in Kilishi and 2.40 His value [29].

The statistics in Table 1 showed a more diversed values when compared with similar type of statistics in Kilishi; this is because the two results ('wara' and Kilishi) had their amino acids profiles divided into EAAs and NEAAs containing nine items in each case. This comparison could be observed to show the disparity ('wara'/Kilishi); EAA: TEAA, 38.0/38.2g/100gcp, mean, 4.22/4.25, SD, 2.03/2.48 and CV%, 48.0/58.4; NEAA: TNEAA, 59.8/52.1g/100gcp, mean, 6.64/5.79, SD, 4.63/3.79 and CV%, 69.7/65.9. Whereas values in 'wara' at EAA comparison, 'wara' was consistently lower than Kilishi but in NEAA 'wara' was consistently higher than Kilishi. As in 'wara' inferential statistic, these values were also low in Kilishi [29]: rxy (0.1549), rxy2 (0.0240) and Rxy (0.2381) which were all correspondingly higher than 'wara' although generally, both samples carried low values of the parameters enumerated. Also, like in 'wara', the CA was high in Kilishi (0.9879) with correspondingly low IFE (0.0121).

As in Kilishi, the value of CA in 'wara' showed that virtually no relationship existed between the Kilishi EAA and NEAA since the error of prediction of relationship was high at 99.2% whereas reduction of error of prediction of relationship was just 0.80%; in Kilishi, CA was 98.79% and IFE was 1.21% [29] similar to the characteristic of 'wara'.

In Table 2, the total amino acid (TAA) level was 97.7g/100gcp. This value was greater than many literature TAAs. 'Wara' TAAs value was greater than (g/100gcp): 90.3 in Kilishi [29]; in nards of heterosexuals of N. maculatus with values of 95.4 – 97.6 [36]; 96.6 – 97.1 in the flesh of the heterosexual N. maculatus [30]; flesh of S. africanus africanus (777.0 mg/gcp) [31]. Column 1 in Table 2 included AAs class and other quality parameters. The total EAA (TEAA) was 42.6 (with His) with a percentage value of 43.6 whereas TNEAA value was 55.1 with a percentage of 56.4. The total aromatic amino acid (TArAA) was 12.8 (13.1%) and its corresponding TEArAA was 9.75g/100gcp (9.98%). 'Wara' was better than Kilishi in these parameters: TArAA(10.4g/100gcp) and 7.32g/100gcp (TEArAA) [29]. The 'wara' TArAA value of 12.8g/100gcp was at the top end of ArAA suggested for ideal protein (68 – 118mg/gcp) [25]; this makes 'wara' to be a good source of ArAA which may also qualify 'wara' as a supplement to foods of lower ArAA values. The 'wara' ArAA of 12.8g/100gcp was much higher than in the flesh of N. masculatus with reported values of 7.72 – 9.67g/100gcp [30]. The (TEAA/TAA)% of 'wara' was 43.6; this is above 39% considered adequate as ideal food for infants, 26% for children and 11% for adults [25]. The TEAA/TAA in egg is 50% [37]. The recommended total sulphur amino acid (TSAA) for infants is 58mg/gcp [25]; this is higher than 3.10g/100gcp in 'wara'.

The constituent of TSAA in the sample was Cys and Met having a grand total of 3.10g/100gcp and 3.17% of total amino acids. The (Cys in TSAA)% was 51.4; this is much far away from the usual animal (Cys/TSAA)% found in literature: 32.8 in Kilishi [29], both innards (26.7 – 33.0) and meat of N. masculatus at 31.9 – 33.1 [36, 30]. 27.3 – 32.8 in S. africanus africanus [31], 36.3 in Macrotermis bellicosus [38]; 25.6 in Zonocerus variegatus [39], 35.5 in Archachatina marginata marginata [40], 38.8 in A. archatina and 21.0 in Limicolaria sp. (the last three examples are land snails consumed in Nigeria) [40]. Some animal %Cys/TSAA had been given in literature, they are rat, chick and pig that their diet must contain 50% as %Cys/TSAA [41], however, such standard value of Cys/TSAA is unknown for man [25]. All the examples given earlier came from animal sources. It should be noted, however, that vegetable protein (e.g coconut meat) has Cys/TSAA of 62.8% [42]. Also literature values of high %Cys/TSAA were in Anacardium occidentale with a value of 50.51 [43]. From all this information, 'wara' had behaved like a vegetable in its (Cys/TSAA)% having a value of 51.4; also it meant that Cys was higher than Met in the sample. 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 [44].

Three types of predicted protein efficiency ratio (P-PER) were calculated and reported in Table 2. They were P-PER1, P-PER2 and P-PER3. The calculated result values were P-PER1 (2.65), P-PER2 (2.48) and P-PER3 (2.41). The in-vivo P-PER is of the order of 2.2 [45]. All the calculated P-PERs were each greater than the in-vivo value; it could be observed also that P-PER1 > P-PER2 > P-PER3. In the series of the P-PER values, 'wara' trend was similar to Kilishi but corresponding values were greater in Kilishi than in 'wara'. This was the trend (P-PER 'wara'/P-PER Kilishi): P-PER1 (2.65/2.70), P-PER2 (2.48/2.62) and P-PER3 (2.41/2.56). The 'wara' P-PER1 and P-PER2 values were lower than the literature P-PER values as follows: N. maculatus meat where values of P-PER1 were 3.39 – 3.69 and P-PER2 were 3.82 – 4.14 [30] and also lower than the report for the innards of N. maculatus heterosexuals with values of P-PER1 (2.83 – 3.01) and P-PER2 (2.89 – 2.96) [36]. Friedman [46] had classified the P-PER values as follows: poor (<1.5), moderate (1.5 – 2.0) and superior (>2.0). On this classification, P-PERs 1, 2, 3 were all in the superior group category. Other literature P-PER values were: meat of female S. africanus africanus, P-PER1 was 3.1 [31]; in Callinectes latimanus (a lagoon crab), P-PER1 was 1.21 and P-PER2 was 1.39 [47]. The P-PER values in 'wara' indicated that it might be a better physiologically utilized protein source than some other animal proteins. On the whole, it has been shown 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. Still in Table 3, we have the Leu/Ile ratio which was 1.74, Leu-Ile (difference) was 3.23 g/100gcp, %Leu-Ile/Leu was 42.7. In Kilishi, Leu/Ile ratio was 1.88, Leu-Ile (difference) was 3.59g/100gcp, %Leu-Ile/Leu was 46.8 (all being higher than in 'wara') [29]. In the meat of N. maculatus Leu/Ile ratio had values of 1.60 – 1.63 (all lower than in 'wara') [30], in meat of S. africanus africanus, the ratio was 1.60 (lower than in 'wara') [31] and the innards of N. maculatus heterosexual, the ratios were 1.54 – 1.91 with differences of 2.87 – 3.97g/100gcp and %(Leu-Ile)/Leu values of 35.1 – 47.7 (all highly comparable to 'wara' values) [36]. The literature value for ideal Leu/Ile is 2.36 [41]. The value of 1.74 was low to 2.36, therefore, we might not experience concentration antagonism in the sample when consumed as protein source in the diet. It has been suggested that an amino acid imbalance from excess Leu might be a factor in the development of pellagra [48]. A high Leu imbalance in the diet impairs the metabolism of Trp and niacin, and is responsible for the niacin deficiency in sorghum eaters [49]. Experiments in dogs had shown that animals fed sorghum proteins with less than 11g/100gcp Leu did not suffer from nicotinic acid deficiency [50]. The present Leu value of 7.57g/100gcp was much lower than 11g/100gcp and therefore considered safe and could be beneficially exploited to prevent pellagra in endemic areas [51].

The essential amino acid index (EAAI) were calculated and reported in two forms which were EAAI1 and EAAI2. The value of EAAI1 corresponded to the value in which soybean was used as standard whereas EAAI2 was the value due to egg standard. EAAI1 in 'wara' was 1.29 better than in defatted soybean flour of 1.26 [52] but lower than in whole hen's egg which is 1.55. Two fancy meats (liver and heart) of African giant pouch rat (Cricetomys gambianus), the EAAI had values of 1.20 – 1.31 [53]. EAAI1 in Kilishi was 1.23 (lower than in 'wara' of 1.29). The quality of 'wara' was further demonstrated by the EAAI2 which was 99.9 with its corresponding biological value of 97.2. In Kilishi EAAI2 was 94.5 with its corresponding BV of 91.3 [29]. Some literature values for EAAI and BV were shown for the purpose of comparison between the values in 'wara' and literature samples, i.e., EAAI2/BV (for 'wara'/literature sample) [22]: 'wara'/milk,cow (whole, nonfat, evaporated or dry), 99.9/88 and 97.2/84 predicted, 90 observed; 'wara'/human, 99.9/87 and 97.2/83; 'wara'/eggs, chicken (whole, raw or dried), 99.9/100 and 97.2/97 predicted, 96 observed; 'wara'/whites (raw or dried), 99.9/95 and 97.2/92 predicted, 93 observed; 'wara'/yolks (raw or dried), 99.9/93 and 97.2/89 (predicted); 'wara'/shellfish (shrimp,

including prawns, raw or canned), 99.9/67 and 97.2/61 (predicted); also 'wara'/meat N. maculatus, 99.9/88.7 – 89.2 and 97.2/85.0 – 85.5 [36]. These literature results exhibited the quality position of 'wara' protein; it showed that except whole hen's egg, the EAAI was better than all literature values. The BV for 'wara' was better than all the literature values. The isoelectric point (pI) calculation from amino acids would assist in the quick production of certain protein isolate of an organic product without evaluating the protein solubility before getting at the pI; the pI of 'wara' was 5.56 showing the sample to be in the acidic medium of the pH range . The pI in Kilishi was 5.63 which is 0.07 (1.24%) greater than in 'wara'.

According to Albanese [54], in infants protein requirements, a growth pattern of amino acid requirements was obtained by assigning a 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 Lys/Trp (L/T) and Met/Trp (M/T) ratios of muscle proteins which constitute about 75% of the infant body proteins. The 'wara' L/T result value was 3.62 and M/T value of 0.948. These results were all lower than these literature values in Kilishi, L/T was 8.55 and M/T was 2.38; in innards of N. maculatus L/T range was 3.00 – 5.01 and meat was 3.31 – 4.27, in the M/T, innards had 1.78 - .50 and meat had 1.97 – 2.64 [30, 36].

Mammalian tissue patterns have the following values: L/T: muscle (6.3), viscera (5.3) and plasma proteins (6.2). M/T: muscle (2.5), viscera (2.0), plasma proteins (1.1) [55]. 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 L/T of 'wara' value was 3.62 which was lower to the standard by 2.68(42.5%); for viscera, result was 1.63(30.8%) lower than the standard; for plasma L/T 'wara' was less than 6.2 by 2.58(41.6%). For M/T, these were observed: muscle, 'wara' was less by 1.55(62.1%), for viscera, 'wara' was less by 1.05(52.6%) and plasma, 'wara' was less by 0.152(13.8%). The present L/T and M/T values were much lower than the values in Kilishi with values of 8.55 (L/T) and 2.38 (M/T) [29]. In N. maculatus innards L/T was 3.00 – 5.0 and meat was 3.31 – 4.27; innards M/T was 1.78 – 3.50 and meat, 1.97 – 2.64 [30, 36]. The mean minimum Phe requirement estimate in the presence of an excess of Tyr is 9.1mg/kg/day. 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 [56]. The Phe/Tyr in the present result was low at 1.85 which did not meet the optimal proportion of dietary Phe and Tyr 60:40 respectively.

The metabolic demand of AAs is for both EAA and NEAA. Due to this, when any or all indispensable amino acids are present in excess of the demand, the absorbed mixture is unbalanced and limited by dispensable AAs. It is assumed that these will be supplied from oxidation of surplus indispensable AAs. However, if such conversion of indispensable to dispensable AAs occurs, then all of the absorbed nitrogen will be utilized in the same way as that of an absorbed mixture which exactly matches the demand (the reference pattern). Based on this, it might be concluded that there can be no benefit from an AA score > 1 with theoretical possibility of a disadvantage if interconversion were incomplete. Table 2 showed that EAA/NEAA had a value of 0.773 showing NEAA to be higher than the EAA; hence, there would be need for interconversion.

In Fig. 3 we have the amino acid groups into classes [26]. The concentration trend of the classes follows as shown in g/100gcp: class IV (27.4) > class I (26.3) > class V (14.2) > class VI (12.8) > class II (10.4) > class VII (6.10) > class III (3.10). In Kilishi, the trend was class I (27.3) (very close to 27.3 in class IV of 'wara' trend) > class IV (22.9) (an interchange with 'wara' trend) > class V (17.1) > class VI (10.4) > class II (7.27) > class VII (4.23) > class III (3.59) [29], that is, classes V, VI, II, VII and III in Kilishi tallied with similar position in 'wara'. In N. maculatus innards, the trend was class I > class IV > class V > class VI > class II > class III > class VII [36]; classes I, IV, III and VII changed position when compared to 'wara' amino acid trend. The trend in N. maculatus meat [30] was similar to the trend in N. maculatus innards [36]. It would also be observed that the percentage values of each amino acid were close to their individual values with slight differences; we have (value/percentage): class I (26.3/26.9); class II (10.4/10.7); class III (3.10/3.17); class IV (27.4/28.0); class V (14.2/14.5); class VI (12.8/13.1); class VII (6.10/6.24). These differences ranged between 0.07 – 0.14. The number of times each type of difference and the overall percentages were: 0.3 (3x = 42.9%); 0.07 (1x =14.3); 0.6 (2x= 28.6); 0.14(1x = 14.3); hence, number times trend: 0.3 (3x) > 0.6 (2x) > 0.07 (1x)≡ 0.14(1x).

Fig. 4 contained the amino acid scores of 'wara' based on whole hen's amino acid profiles. On this standard, 'wara' had scores greater than 1.00 : Phe (1.10), His (1.08), Gly (1.62), Ser (1.61) and Asp (1.44) whereas in Kilishi, such amino acids were Lys, His, Gly, Pro, Ala and Glu [29]. In 'wara', the limiting amino acid (LAA) was Met(0.459); this is unusual as most animal LAA used to be Ser. Examples: in Kilishi, Ser was LAA with a value of 0.46 (close to Met of 0.459 in 'wara') [29], Ser was limiting in male (0.511) and female (0.487) in the innards of N. maculatus, also in the meat of N. maculatus Ser was limiting in male (0.513) and in female (0.516) [30, 36]. For the correction of the LAA score to bring to normal level of amino acid profile in order to fulfil the day's needs for all the amino acids in the 'wara' sample, it is 100/45.9 = 2.18 times as much 'wara' protein would have to be consumed (eaten) when it serves as the sole protein source in the diet. The value of 2.18 was close to the value of 2.17 in Kilishi [29]. In Fig. 5, we have EAA scores of 'wara' based on FAO/WHO [24] standards. All the EAAs and the total amino acids scores with exception of Lys, had scores > 1.00. It meant that the limiting amino acid under this standard was Lys (LAA=0.966). Val was LAA in Kilishi with value of 0.882. Correction for normal amino acid profile would be 100/96.6=1.04, that is, 1.04 x protein value for full protein availability for body use. In Table 7, we have the EAA scores of 'wara' based on requirements of pre-school child (2-5y) requirement score comparison [25]. As we have in Fig. 5, the LAA in Fig. 6 was also Lys with a value of 0.97. In the corection for total required amino acids, it was 100/97.0 or 1.03x 'wara' protein. Trp (0.927) was LAA in Kilishi [29].

The calculated values of the estimates of amino acid requirements at ages 10-12 years in mg/kg/day at the body weight of 30kg were depicted in Fig. 7. The protein of the 'wara' sample had values greater than the estimates in all the amino acids to the tune of 3.72 – 330%. In the four major limiting amino acids of Lys (first), Met + Cys (second), Thr (third) and Trp (fourth), the percentage 'wara' protein excess values were: Lys (3.72%), Met + Cys (27.7%), Thr (40.3%) and Trp (330%). All these values for 'wara' were far lower when compared to Kilishi values [29] as shown, amino acid ('wara'/Kilishi value): Lys (3.72/211%), Met + Cys (27.7/185%), Thr (40.3/123%) and Trp (330/448%). In 'wara' concentration value follows this trend among the four EAAs on this section of discussion: Lys < Met + Cys < < Thr < Trp whereas in Kilishi, the trend was: Thr < Met + Cys < Lys < Trp [29].

The amino acids in the body generally function in different ways for the body to maintain its homeostasis. Phenyalanine is a precursor for neurotransmitters which help in the production of other amino acids and their functioning. Valine is involved in the stimulation of muscle growth, regeneration and it is also involved in energy production. Threonine (number three among the four major essential amino acids) is a principal component of structural proteins such as collagen and elastin which are present in skin and connective tissues, in fat metabolism and immune function. Tryptophan (number four of the first four essential amino acids) is a precursor to serotonin production, a neurotransmitter that helps in appetite, sleep and mood regulation. Met (number two of the first four essential amino acids in conjunction with Cys) 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 assists in regulating blood sugar levels, enhances wound healing, haemoglobin production and energy regulation. Branched chain amino acids are Val, Leu and Ile. Lys helps in protein synthesis, calcium absorption, immune function, energy production, hormone production and in collagen production. Histidine, a neurotransmitter helps myelin sheath that surround the nerve cells, helps in digestion, immune response, sleep-wake cycles and sexual functions [58].

Conclusion

'Wara' (Nigerian Local Cheese) would serve as a good source of animal protein of high quality amino acids. The crude protein was high at 33.4g/100g. The two highest amino acids had values of 17.3g/100gcp (Glu) and 10.1g/100gcp (Asp) and both are acidic amino acid and the third highest was Leu (7.57g/100gcp) which is an essential amino acid. The amino acids into essential and nonessential amino acid groups showed no significant difference at r=0.01. The quality parameters showed the EAAs as totaling 42.6g/100gcp and percentage of 43.6. The TSAA was 3.10g/100gcp but the %Cys/TSAA was 51.4 (which is a characteristic value of plants). Leu/Ile ratio of 1.74 is a good nutritional value. The followings show the bioavailability of 'wara': P-PER1 – P-PER3 (2.65 – 2.41) (all in the superior group), EAAI1 (1.29, soybean standard), EAAI2 (99.9, egg standard) and BV (97.2). The amino acids group values (g/100gcp) and their individual percentage values were very close with difference range of 0.07 – 0.14%. Whereas the amino acid score due to egg comparison had limiting amino acid of Met (0.459), those of EAA scoring pattern and the pre-school requirements had respective scores of 0.966 and 0.97, both being due to Lys. For the estimates of amino acid requirements at ages 10-12 years (mg/kg/day) 'wara' had values of each EAA being higher than the recommended values. With all the above information, 'wara' would serve as a healthy protein source with a lot of health benefits, it is protein dense, can be consumed in various forms and kept with minimum effort after preparation to consumption.

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