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Relationship in Amino Acid Quality between Raw, Steeped and Germinated Wheat (*Triticum durum*) Grains

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Abstract

Levels of amino acids were determined in the grains of wheat, Triticim durum Desf. The raw sample was best in Arg, Ile, Leu, Thr, Val, Ala, Asp, Glu and Pro but similar to germinated sample in Lys whereas germinated sample was best in His, Met, Cys, Phe,Gly, Ser and Tyr. The total amino acid contents were; raw 12[74.1 g/100 g crude protein (c. p.)], steeped 63.4 g/100g c. p.) and germinated (70.6 g/100 g c. p.) with respective essential amino acids of 32.9 g/100 g c. p., 30.0 g/100 g c. p. and 32.4 g/100 g c. p. The Predicted Protein Efficiency Ratio (P-PER) levels were 2.46 (raw), 2.27 (steeped) and 2.19 (germinated). Only germinated samples enhanced the quality of the wheat amino acid levels up to the tune of 41.2 %. However, significant differences occurred between raw/steeped and raw/germinated at $r = _{0.05}$.

Key words: , Amino acid, Raw, Steeped, Germinated, Wheat grains

Introduction

Wheat is the world's most widely cultivated plant. It is grown from the equator to 60° N and 40° S, with the greatest concentration in the warm temperate regions where the winters are cool followed by relatively dry and warm summers for ripening and with moderate rainfall between 30 and 90 cm (Kochhar, 1986). All wheats, whether wild or cultivated belong to the genus *Triticum* of the Tribe Triticeae in the family Poaceae and sub family Poaideae (Kochhar, 1986), of all the wheats, bread wheat; *T. aestivum* L. is by far the most important and widely grown food crop. *T. aestivum* is hexaploid wheat. Some varieties of *Triticum durum* Desf, used for making macaroni, have yielded well in Nigeria (Phillips, 1977). *T. durum* is tetraploid wheat (Willis, 1973). It is called *alakama* both in Yoruba and Hausa (Phillips, 1977).

The uses of wheat in the baking industry have been enumerated particularly in *T. aestivum* but little is known about the composition and food properties of *T. durum*. Published works on *T. durum* included the determination of the levels of phytate, phytate/zinc (Phy/Zn), calcium/phytate (Ca/Phy) and calcium/ phytate/zinc ([Ca][Phy]/[Zn] (Adeyeye et al, 2000); determination of chemical composition and the effect of salts on the food properties of *T. durum* whole meal flour (Adeyeye and Aye, 2005); determination of amino acids and sugar composition of *T. durum* whole meal flour (Adeyeye, 2007). Often, sorghum (*Sorghum bicolor*), millet (*Pennisetum typhoides*) and corn (*Zea mays*) are steeped, wet-milled and fermented to prepare ogi as weaning food gruel for babies. [Ogi is a smooth, free-flowing thin porridge obtained from wet-milled, fermented corn, sorghum or millet.]

This work reports on the amino acid composition of the raw, steeped and germinated grains of *Triticum durum*. This will give information on the best treatment to enhance the protein quality for its various food uses.

Materials and Methods

The wheat grains were collected from farms located in Zaria, Kaduna State, Nigeria. About 1.5 kg of the grains was used for the experiments. First of all, the shaft was removed by threshing, sorting of grains was done to separate bad grains, stones and other non -wheat particles. The sample was divided into three equal parts for use as raw, steeped and germinated sample and labeled accordingly.

Sample treatment

The portion labeled as raw (0.5 kg) was not specially treated but was only dried to constant weight (8.87g/100 g moisture content). The portion labeled for steeping (0.5 kg) was

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placed in a plastic container, distilled water poured to cover the grains and left in the laboratory at ambient temperature (30.9 °C) at 0.41 Im^2/ft light intensity for four days. After this, the grains were washed with distilled water, dried in the sun to constant weight (9.56 g/100 g moisture content) and stored in a covered plastic container. The portion labeled for germination (0.5 kg) was treated as follows: grains were soaked in distilled water at room temperature for 24 h; the grains were then spread on a damp fabric, protected from the direct sun for approximately 48 h, until 5.04 cm sprouts developed; germinated grains were dried in the sun for three days until constant weight of 8.94 g/100 g moisture content; the sprouts were manually removed and the desprouted grains were stored in a plastic container (WHO, 1999). Each sample was then homogenized, sieved using a 200 mm mesh and kept in the refrigerator (-4 °C) pending analysis. Six replicates of steeped and germinated grains were used.

Determination of amino acids

The amino acid profile in the known sample was determined using methods described by Spackman *et al* (1958). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-sample Amino Acid Analyzer (TSM).

Wheat flour (2.0 g) of each group was defatted with chloroform/methanol of 100 ml/50 ml (2:1v/v) mixture using the Soxhlet apparatus with total extraction mixture of 150 ml at a boiling range 61.7- 65.0 °C (AOAC, 2005). Extraction of lipid continued for at least 5 h to ensure total lipid extraction. Between 30-35 mg of the defatted sample was put in a glass test tube,7 ml of 6 M HCl was added and oxygen expelled by flushing with nitrogen gas. The sealed test tube was put in an oven at 105±5 °C for 22 h and later allowed to cool before the content was filtered. The filtrate was evaporated to dryness at 40 °C under vacuum in rotary evaporator .The residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer. The amount loaded was between 5-10 microlitres. This was dispensed into the cartridge of the analyzer. Determinations were in duplicate. The period of an analysis lasted for 76 min for each sample. The column gas flow rate was 0.50 ml/min at 60 °C with reproducibility within ± 3 % using norleucine as the internal standard.

Method of calculating amino acid values from the chromatogram peaks

The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half- height of the peak on the chart was found and the width of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the width at half-height. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

NE = Area of norleucine peak
Area of each amino acid

A constant S was calculated for each amino acid in the standard mixture:

$S_{std} = NE_{std} \times \text{mol. Weight} \times \mu\, MAA_{std}$

Finally the amount of each amino acid present in the sample was calculated in g/16 N or g/100 g protein using the following formula:

Concentration (g/100 g protein) = $NH \times W@NH/2xS_{std} \times C$

Where: C Dilution x 16/sample wt (g) x n%x 10 vol. loaded ÷ NH x W (nleu)

Where: NH = Net height
W = Width @half-height
Nleu = Norleucine.

Estimation of isoelectric point (pI):

The estimation of pI for a mixture of amino acid was calculated using the equation of Finar (1975)

$$IPm = \sum_{i=1}^{n} IPiXi$$

where IPm is the isoelectric point of amino acid, IPi is the isoelectric point of the ith amino acid in the mixture and Xi is the mass or mole fraction of the ith amino acid in the mixture (Olaofe and Akintayo 2000).

Estimation of quality of dietary protein

The total amino acids scores were calculated based on the whole hen's egg amino acid profile (Paul *et al,* 1976) whilst the essential amino acids scores were calculated using the following formula (FAO/WHO, 1973):

Amino acid score = Amount of amino acid per test protein [mg/g] Amount of amino acid per protein in reference pattern [mg/g].

Also calculated were essential amino acid scores based on the suggested pattern of essential amino acid requirements of pre-school child (2-5 years) (FAO/WHO/UNU, 1985).

The predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer *et al* (1974); i.e.: P-PER = -0.468 + 0.454 (Leu) -0.105 (Tyr).

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 according to Steinke *et al* (1980):

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

Determination of the ratio of total amino acids (TEAA) to the total amino acids (TAA), i.e. (TEAA/TAA), total sulphur amino acid (TSAA), percentage cystine in TSAA (% Cys/TSAA), total aromatic amino acid (TArAA), total neutral amino acid (TNAA), total acidic amino acid (TAAA) and total basic amino acid (TBAA) were estimated from the amino acids profile. The leucine/isoleucine ratios were calculated.

Statistical analysis

All data generated were analyzed statistically (Oloyo, 2001). Calculated for were the grand mean, standard deviation (SD) and co-efficient of variation in percent (CV %). Also calculated were linear correlation coefficient (r_{xy}), degree of association $(r_{xy})^2$, linear regression (Rc), coefficient of alienation (C_A) and index of forecasting efficiency percent (IFE %). The r_{xy} was subjected to table - r at $r_{=0.05}$ at n-2 degrees of freedom where n is the number of data items. The calculated were meant to determine the levels of variation among the data obtained for raw, steeped and germinated samples and to determine if significant differences existed between raw/ steeped and between raw/germinated in the amino acid composition and amino acid scores.

Results and Discussion

Table I shows the amino acid composition of the samples. In most of the results on pair wise basis, the values for the raw were all better than the values in the steeped samples. On the other hand, levels of His, Met, Phe, Cys, Gly, Ser and Tyr in germinated samples were correspondingly higher than those in raw and steeped, meaning that germinated sample was 41.2 % best in 7 parameters (7/17) of the amino acids than in steeped but 43.8 % best in 7 parameters (7/16) of the amino acids than in raw because in both raw and germinated samples, Lys value was similar in both with a value of 4.16 g/100 g crude protein (c. p.). This trend between raw and germinated wheat flours amino acid profiles was also observed between raw and germinated millet (*Pennisectum typhoides*) where 60 % was best in 9 parameters (9/15) in the germinated than the raw whereas Cys also shared similar values of 7.6 mg/g c. p. between raw and germinated millet flours (Adeyeye, 2009a).The protein level of steeped sample was highest (14.125 or 14.1 g/100 g) > raw (14.0625 or 14.1 g/100 g) > germinated (13.4375 or 13.4 g/100 g). The results in the wheat flours were in contrast to the observation in sorghum where the steeped samples had the best levels of amino acids (AA) in His, Arg, Thr, Ser, Pro, Gly, Ala, Met, Cys, Val, Phe and Tyr, almost like the germinated samples of wheat (Adeyeye, 2008a).The highest AA was Glu with values (g/100 g c. p.) of 14.2, 9.10 and 13.0 for raw, steeped and germinated grains, respectively. Tryptophan was not determined. Glu also had the highest levels in sorghum samples (Adeyeye, 2008a) and millet samples (Adeyeye 2009a). Leucine was the highest concentrated essential AA (EAA) in raw (7.12 g/100 g c. p.), steeped (6.66 g/100 g c. p.) and germinated (6.58 g/100 g c. p.) samples. Leu was also highest in millet samples but Ile was the highest concentrated EAA in sorghum samples. The least varied AA was Tyr which ranged from 2.70 g/100 g c. p. (steeped) to 3.17 g/100 g c. p. (germinated) with coefficient of variation percent (CV %) of 0.08 whilst the most varied AA was Cys with values of 0.70 g/100 g c .p. (steeped), 1.0 g/100 g c. p. (raw) and 1.10 g/100 g c. p. (germinated) with CV % of 22.4. Results in wheat flour EAA were better than in the sorghum in His, Leu, Lys, Met, Phe and Val (Adeyeye, 2008a). Even though the Lys contents in the samples (3.91- 4.16 g/100 g c. p. or 39.1-41.6 mg/g c .p.) were lower than the Lys content of the reference egg protein (62 mg/g c. p.), they can be enhanced by mixing with legumes, which are high in Lys. The increase in AA content of germinated wheat grains might be due to increase

Table I: Amino Acid (g/100 g Crude Protein) Composition of Raw, Steeped and Germinated Wheat (on Dry Weight)

Amino acid	Raw	Steeped	Germinated	Mean	SD	CV%
Arginine (Arg)*	4.66	4.0	3.62	4.09	0.53	12.9
Histidine (His)*	2.24	2.15	2.30	2.23	0.08	3.39
Isoleucine (Ile)*	3.55	3.14	3.23	3.31	0.22	6.51
Leucine (Leu)*	7.12	6.66	6.58	6.79	0.29	4.29
Lysine (Lys)*	4.16	3.91	4.16	4.08	0.14	3.54
Methionine (Met)*	0.90	0.83	1.04	0.92	0.11	11.6
Phenylalanine (Phe)*	3.60	3.43	4.30	3.78	0.46	12.2
Tryptophan (Try)*	-a	-	-	-	-	-
Threonine (Thr)*	3.25	2.65	2.76	2.89	0.32	11.1
Valine (Val)*	4.16	3.84	3.72	3.91	0.23	5.82
Alanine (Ala)	4.14	3.65	3.03	3.61	0.56	15.4
Aspartic acid (Asp)	9.34	8.87	8.93	9.05	0.26	2.82
Cystine (Cys)	1.00	0.70	1.10	0.93	0.21	22.4
Glutamic acid (Glu)	14.2	9.10	13.0	12.1	2.67	22.0
Glycine (Gly)	3.34	3.12	3.70	3.39	0.29	8.64
Proline (Pro)	3.05	2.34	2.64	2.68	0.36	13.3
Serine (Ser)	2.55	2.33	3.36	2.75	0.54	19.7
Tyrosine (Tyr)	2.90	2.70	3.17	2.92	0.24	0.08
Protein	14.1	14.1	13.4	13.9	0.40	2.91

* Essential amino acid; SD = standard deviation; CV = coefficient of variation; [a] = not determined

in the protease activity of enzymes which break down the protein to release amino acids needed for the activity.

Table II shows several quality parameters of protein in the samples. The EAA ranged between 30.0 g/100 g c. p. to 32.9 g/100 g c. p. These values were far from the value of 56.6 g/100 g c. p. of the egg reference protein (Paul *et al,* 1976) but slightly close to 45.3 g/100 g c. p. for peanut meal (Lusas, 1979) and better than 19.0 g/100 g c.p. for *Colocynthis citrulus* flours (Akobundu *et al,* 1982); 21.5-30.7 g/100 g c. p. in sorghum grains (Adeyeye, 2008a) and 21.0-23.4 g/100 g c.p. in millet grains (Adeyeye, 2009a).The total sulphur AA (TSAA) of the samples were 1.90, 1.53 and 2.14 g/100 g c. p. for raw, steeped and germinated, respectively, these values were individually about 32.8 % (raw), 26.4 % (steeped) and 36.9 % (germinated) of the value of 5.8 g/100 g c .p. recommended for infants (FAO/WHO/UNU, 1985). The aromatic amino acid (ArAA) range suggested for ideal infant protein (6.8-11.8 g/100 g c. p.) (FAO/WHO/UNU, 1985) is higher than the current report (6.13-7.47 g/100 g c .p.) indicating that when wheat should be used to prepare gruel as the weaning food, it should be complemented with ArAA rich foods particularly when raw or steeped wheat grain is used. The percentage ratio of EAA to TAA in the flour ranged from 44.4 - 47.3. These values were well above the 39 % considered to be adequate for ideal protein food for infants, 26 % for children and 11 % for adults (FAO/WHO/UNU, 1985). The percentages of EAA/TAA for the wheat samples could be favourably compared with that of egg (50 %) (FAO/WHO, 1990), pigeon pea flour (43.6 %) (Oshodi *et al,* 1993), beach pea protein isolates (43.8-44.4 %) (Chavan *et al,* 2001), 53.1-56.7 % in sorghum flours (Adeyeye, 2008a). The P-PER as shown in Table II ranged from 2.19-2.46. The experimentally determined PER usually ranged from 0.0 for a very poor protein to a maximum possible of just over 4 (Muller and Tobin, 1980). In the samples (as shown in Table1) it could be seen that the values for Leu and Tyr (from which P-PER were calculated) were almost half of each other raw (7.12 and 2.90 g/100 g c.p. respectively), (6.6 and 2.70 g/100 g c.p. respectively) and germinated (6.58 and 3.17 g/100 g c. p. respectively). The P-PER in wheat grains were: raw, steeped (2.46), steeped (2.27) and germinated (2.19) meaning that the physiological utility in the body of wheat grains would be much better than the sorghum (with P-PER of 0.0 - 0.29) (Adeyeye, 2008a) and in millet (with P-PER of 1.32-1.66) (Adeyeye, 2009a).

Table II: Total, Essential, Non-Essential, Neutral, Acidic, Basic, Sulphur, Aromatic Acid (g/100 g Crude Protein). Protein Efficiency Ratio (P-Per), Isoelectric Point (pI), Leu/Ile Ratio, Leu/Ile Difference of Wheat (on Dry weight)

Amino acid	Raw	Steeped	Germinated	Mean	SD	CV%
TAA[a]	74.1	63.4	70.6	69.4	5.46	7.86
TNEAA[b]	41.2	33.4	38.2	37.6	3.93	10.5
TEAA[c]						
-with His	32.9	30.0	32.4	31.8	1.55	4.88
-no His	30.6	27.9	30.1	29.5	1.44	4.87
%TNEAA	55.6	52.7	54.2	54.2	1.45	2.68
%TEAA						
-with His	44.4	47.3	45.8	45.8	1.45	3.17
-no His	41.3	43.9	42.6	42.6	1.3	3.05
TNAA[d]	39.6	35.4	38.6	37.9	2.19	5.79
%TNAA	53.4	55.8	54.7	54.6	1.20	2.20
TAAA[e]	23.5	18.0	21.9	21.1	2.83	13.4
%TAAA	31.7	28.3	31.0	30.3	1.80	5.93
TBAA[f]	11.1	10.1	10.1	10.4	0.58	5.55
%TBAA	14.9	15.9	14.3	15.0	0.81	5.39
TSAA	1.90	1.53	2.14	1.86	0.31	16.5
%TSAA[g]	2.56	2.41	3.03	2.67	0.32	12.1
%Cys inTSAA	52.6	45.8	51.4	49.9	3.63	7.27
TArAA[h]	6.50	6.13	7.47	6.7	0.69	10.3
%TArAA	8.77	9.67	10.6	9.68	0.92	9.45
P - PER	2.46	2.27	2.19	2.31	0.14	6.06
Leu/Ile	2.01	2.12	2.04	2.06	0.06	2.76
Leu-Ile (diff.)	3.57	3.52	3.35	3.48	0.12	3.31
%Leu-Ile (diff)	50.1	52.9	50.9	51.3	1.44	2.81
pI (calculated)	4.09	3.58	3.88	3.85	0.26	6.66
EAAI[i]	1.01	0.90	0.98	0.96	0.06	5.92

[a] = total acid; [b] = total non- essential amino acid; [c] = total essential amino acid; [d] = total neutral amino acid; [e] = total acidic amino acid; [f] = total basic amino acid; [g] = total sulphur amino acid; [h] = total aromatic amino acid; [i] = essential amino acid index; pI = isoelectric point.

The Leu/Ile values ranged as follows: 2.01 (raw), 2.12 (steeped) and 2.04 (germinated). In all the samples (Table I) the level of Leu was more than twice the level of Ile in each wheat sample. Endemic pellagra in cereal- eating populations was first described by Gopalan and Srikantia (1960) particularly in poor agricultural labourers around Hyderabad in Andhra Pradesh (India). It has been suggested that an amino acid imbalance from excess Leu might be a factor in the development of pellagra (FAO, 1995). High Leu in the diet impairs tryptophan and niacin metabolism and is responsible for niacin deficiency in sorghum eaters (Balavady *et al,* 1963) and hence, the hypothesis that excess Leu in sorghum is aetiologically related to pellagra in sorghum- eating populations (FAO, 1995). The study of Krishnaswamy and Gopalan (1971) had suggested that the Leu/Ile balance is more important than dietary excess of Leu alone in regulating the metabolism of Try and niacin and hence the disease process. However, it has also been suggested that factors other than excess Leu and poor Leu/Ile balance in cereal proteins are responsible for the development of the disease. Krishnaswamy *et al* (1976) have shown that vitamin B6 is involved in the metabolism of Leu as well as that of Try and niacin suggesting that of pellagra might be related to the nutritional status of population in terms of vitamin B_6. Experiments in dogs have shown that animals fed sorghum proteins with less than 11.0 g/100 g c.p. Leu did not suffer from nicotinic acid deficiency (Belavady and Udayasekhara Rao, 1979). The current report shows Leu to range from 6.58-7.12 g/100 g c. p. which was far less than 11.0 g/100 g c. p., therefore, samples are considered safe and could be beneficially exploited to prevent pellagra in endemic areas (Deosthale, 1980). Table II shows that the % Cys in TSAA ranged from 45.8-52.6. Cys can spare with Met in improving protein quality and has positive effects on mineral absorption, particularly zinc (Mendoza, 2002). % Cys/TSAA obtained in this study for raw (52.6) and germinated (51.4) were comparable to the value of 62.9 reported for coconut endosperm (Adeyeye, 2004), this stands a good chance in carrying out its functions effectively. The % Cys in TSAA in sorghum ranged from 58.9-72.0 (Adeyeye, 2008a); it is 50.5 % in cashew nut (Adeyeye *et al,* 2007). On the other hand, the % Cys/TSAA in the steeped wheat sample was 45.8 like in *Triticum durum* whole meal flour with a value of 40.7 (Adeyeye, 2007), 44.4 in *Parkia biglobosa seeds* (Adeyeye, 2006), 44.3 in Cola acuminata and 37.8 in Garcinia kola (Adeyeye et al, 2007). Most animal proteins are low on Cys and hence in % Cys/TSAA: 36.3 in *Macrotermes bellicosus* (Adeyeye, 2005a); 25.6 in *Zonocerus variegatus* (2005b); 35.3 in *Archachatina marginata*, 388 in *Archatina archatina* and 20.0 in Limicolaria sp. respectively (Adeyeye and Afolabi, 2004); 27.3-32.8 in various parts of west African fresh water female crab (Adeyeye, 2008b) The % Cys/TSAA had been set at; 23.8-30.1 in three different fresh water fish samples (Adeyeye, 2009b). Thus for animal protein, Cys is unlikely to contribute up to 50 % of the TSAA (FAO/WHO, 1991). The % Cys TSAA had been set at 50 % in rat, chick and pig diets (FAO/WHO, 1991). The calculated isoelectric point (pI) ranged from 3.58-4.09.

The calculated pI in *T. durum* in previous work (wheat whole meal flour) was 4.0 (Adeyeye, 2007) whereas the experimental value in the same sample was pH 5 (Adeyeye and Aye, 2005), these values were close to the current raw wheat flour pI of 4.09. The total acidic amino acid (TAAA) about

Table III: Amino acid composition differences between raw and steeped, between raw and germinated wheat samples

Amino acid	Raw-steeped	Raw-germinated	Mean	SD	CV%
Arg	0.66(14.2)	1.04(22.3)	0.85	0.27	31.6
His	0.09(4.02)	- 0.06(2.68)	0.08	0.02	2.8
Ile	0.41(11.5)	0.32(9.01)	0.37	0.06	17.4
Leu	0.46(6.46)	0.54(7.58)	0.5	0.06	11.3
Lys	0.25(6.01)	0.0(0.0)	-	-	-
Met	0.07(7.78)	-0.14(15.6)	0.11	0.05	47.1
Phe	0.17(4.72)	-0.7(19.4)	0.44	0.37	86.2
Thr	0.6(18.5)	0.49(15.1)	0.55	0.08	14.3
Try-	-	- -	-		
Val	0.32(7.69)	0.44(10.6)	0.38	0.08	22.3
Ala	0.49(11.8)	1.11(26.8)	0.8	0.44	54.8
Asp	0.47(5.03)	0.41(4.39)	0.44	0.04	9.64
Cys	0.3(30)	-0.1(10)	0.2	0.14	70.7
Glu	5.1(35.9)	1.2(8.45)	3.15	2.76	87.5
Gly	0.22(6.59)	-0.36(10.8)	0.29	0.10	34.1
Pro	0.71(23.3)	0.41(13.4)	0.56	0.21	37.9
Ser	0.22(8.63)	-0.81(31.8)	0.52	0.42	81.0
Tyr	0.2(6.90)	-0.27(9.31)	0.24	0.05	21.1
Protein	-0.0625(0.44)	0.625(4.44)	0.34	0.40	116

*= figures in brackets are all percentage values; ** = a figure preceded by '-'means both the figure and the figure in bracket carries it.

doubled the total basic amino acid (TBAA) for each sample as shown (TAAA/TBAA): 23.5/11.1 (raw), 18.0/10.1 (steeped) and 21.9/10.1 (germinated) and these were almost reflected in the pI values. The information on pI is a good starting point in predicting the pI for proteins in order to enhance a quick precipitation of protein isolate from biological samples.

The essential amino acid index (EAAI) ranged from 0.90-1.01 which were lower than the value of 1.26 in defatted soy flour (Cavins *et al,* 1972) but better than in millet grain flours with a range of 0.61-0.70 (Adeyeye, 2009a). The EAAI method can be useful as a rapid tool to evaluate food formulations for protein quality (Nielsen, 2002).

Table III contains a summary of the differences between raw/steeped and between raw/ germinated samples. The highest CV % was observed in protein with a value of 116 whereas Lys had a CV % of 0.0. Table III gave all the differences in amino acid parameters.

Table IV shows the amino acid scores based on whole hen's egg. Table IV shows that Met was the limiting amino acid in all the samples with values ranging from 0.26 (26 %)- 0.33

Table IV: Amino acid scores of the samples rased on whole hen's egg amino acid profile

Amino acid	Raw	Steeped	Germinated	Mean	SD	CV%
Arg	0.76	0.67	0.59	0.67	0.09	12.7
His	0.93	0.90	0.96	0.93	0.03	3.23
Ile	0.63	0.56	0.58	0.59	0.04	6.11
Leu	0.86	0.80	0.79	0.82	0.04	4.62
Lys	0.67	0.63	0.67	0.66	0.02	3.50
Met	0.28	0.26	0.33	0.29	0.04	12.4
Phe	0.71	0.67	0.84	0.74	0.09	12.0
Thr	0.64	0.52	0.54	0.57	0.06	11.3
Try	-	-	-	-	-	-
Val	0.55	0.51	0.50	0.52	0.03	5.09
Ala	0.77	0.68	0.56	0.67	0.11	15.7
Asp	0.87	0.83	0.83	0.84	0.02	2.75
Cys	0.56	0.39	0.61	0.52	0.12	22.2
Glu	1.18	0.76	1.08	1.01	0.22	21.7
Gly	1.11	1.04	1.23	1.13	0.10	8.50
Pro	0.80	0.62	0.69	0.70	0.09	13.0
Ser	0.32	0.29	0.43	0.35	0.07	21.1
Tyr	0.73	0.68	0.79	0.73	0.06	7.54
Total	0.76	0.65	0.72	0.71	0.06	7.84

Table V: Amino acid scores of the wheat samples based on the provisional amino acid scoring pattern

Amino acid	Raw	Steeped	Germinated	Mean	SD	CV%
Ile	0.89	0.79	0.81	0.83	0.05	6.38
Leu	1.02	1.01	0.94	0.99	0.04	4.40
Lys	0.76	0.71	0.76	0.74	0.03	3.90
Met +Cys (TSAA)	0.54	0.44	0.61	0.53	0.09	16.1
Phe + Tyr (TArAA)	1.08	1.02	1.25	1.12	0.12	10.7
Thr	0.81	0.66	0.69	0.72	0.08	11.0
Try	-	-	-	-	-	-
Val	0.83	0.77	0.94	0.85	0.09	10.1
Total	0.88	0.80	0.86	0.85	0.04	4.9

(33 %), other AA scores less than 50 % were Ser (32 %, raw); Cys (39 %) and Ser (29 %) in steeped and Ser (43 %, germinated). In order to correct for the whole amino acids profile in the samples, 100/28, 100/26 and100/33 or 3.57, 3.85 and 3.03 times as much raw, steeped and germinated protein would have to be eaten when they serve as sole protein source in the diet respectively (Bingham, 1977).

Table V contains the essential amino acid scores of the wheat grain samples based on EAA scoring pattern (FAO/WHO, 1973) which shows that Met + Cys (TSAA), 0.54 or 54.0 % was the limiting EAA in raw, Met +Cys (0.44 or 44%) was the limiting EAA in steeped whilst Met + Cys (0.61 or 61%) was also the limiting EAA in germinated samples. Therefore, in order to fulfill the day's needs for all the EAA in wheat sample flours, 100/54 (raw), 100/44 (steeped) and100/61 (germinated) or 1.85, 2.27 and 1.64 times as much raw, steeped and germinated wheat protein would have to be eaten when it is the only protein source in the diet. The scores in Table IV and V showed that the wheat samples were better in protein quality than the millet samples (Adeyeye, 2009a) as well as the sorghum samples (Adeyeye, 2008a).

Table VI contains the amino acid scores of the wheat samples based on the EAA suggested pattern of requirements by

Table VI: Amino acid scores of the wheat samples based on the suggested patterns of amino acid requirements for pre-school child (2-5 Years)

Amino acid	Raw	Steeped	Germinated	Mean	SD	CV%
His	1.18	1.13	1.21	1.17	0.04	3.45
Ile	1.27	1.12	1.15	1.18	0.08	6.73
Leu	1.08	1.01	1.0	1.03	0.04	4.23
Lys	0.72	0.67	0.72	0.70	0.03	4.12
Met +Cys (TSAA)	0.76	0.61	0.86	0.74	0.13	17.0
Phe +Tyr (TArAA)	1.03	0.97	1.19	1.06	0.11	10.7
Thr	0.96	0.78	0.81	0.85	0.10	11.3
Try	-	-	-	-	-	-
Val	1.19	1.10	1.06	1.12	0.07	5.94
Total	1.00	0.91	0.99	0.97	0.05	5.09

Table VII: Statistical summary of Tables I, II (pI only), III, IV and V

Correlated parameter	r_{xy}	r_{xy2}	Rc	Slope	CA	IFE	n^{-2}	r-value	Remark
1. Raw/steeped(R/S)	0.9608	0.92	0.23	0.83	0.28	72	16	0.468	*
Raw/germinated(R/G)	0.9911	0.98	0.19	0.91	0.14	86	16	0.468	*
2.(pIonly)Raw/steeped(R/S)	0.9666	0.93	1.14	0.83	0.26	74	15	0.482	*
Raw/germinated(R/G)	0.9620	0.93	3.23	0.81	0.26	74	15	0.482	*
3. Raw-steeped/Raw-germinated	0.5513	0.30	0.43	0.16	0.84	16	15	0.482	*
4. Raw/steeped(R/S)	0.9122	0.83	0.06	0.80	0.41	59	16	0.468	*
Raw/germinated (R/G)	0.9077	0.82	0.05	0.90	0.42	58	16	0.468	*
5. Raw/steeped (R/S)	0.9800	0.96	-0.17	1.11	0.20	80	7	0.666	*
Raw/germinated(R/G)	0.8531	0.73	0.01	1.01	0.52	48	7	0.666	*
6. Raw/Steeped(R/S)	0.9683	0.94	0.11	1.01	0.24	76	7	0.666	*
Raw/germinated	0.8260	0.68	0.22	0.76	0.57	43	7	0.666	*

r_{xy} = linear correlation coefficient; C_A = coefficient of alienation; IFE = index forecasting efficiency,
n = degree of freedom; *values significantly different at $r = _{0.05}$

Table VIII: Summary of the amino acid profile into factors A and B

	Wheat samples (Factor A)			Factor B Means
	Raw	Steeped	Germinated	
Amino acid composition (Factor B)				
Total essential amino acid	32.9	30.0	32.4	31.8
Total non- essential amino acid	41.2	33.4	38.2	37.6
Factor A means	37.1	31.7	35.3	43.7

Table IX: The amino acid profiles of wheat, pearl millet and guinea corn compared

Amino acid	Raw			Steeped			Germinated		
	Wheat	Millet	Sorghum	Wheat	Millet	Sorghum	Wheat	Millet	Sorghum
Arg	46.6	29.4	31.9	40.0	33.6	42.8	36.2	31.0	39.4
His	22.4	15.8	16.5	21.5	16.2	22.9	23.0	18.1	18.1
Ile	35.5	23.1	46.2	31.4	17.8	50.9	32.3	20.1	56.0
Leu	71.2	51.2	10.9	66.6	43.7	21.3	65.8	49.5	21.4
Lys	41.6	20.6	14.8	39.1	20.0	18.8	41.6	21.7	20.0
Met	9.0	6.7	6.2	8.3	5.9	8.0	10.4	7.0	7.0
Phe	36.0	27.7	21.6	34.3	26.0	26.0	43.0	29.4	23.4
Thr	32.5	20.1	22.4	26.5	17.7	32.3	27.6	22.1	30.2
Try	-	-	-	-	-	-	-	-	-
Val	41.6	29.6	18.5	38.4	29.0	38.1	37.2	33.9	30.3
Ala	41.4	18.5	12.9	36.5	21.0	43.9	30.3	23.0	20.1
Asp	93.4	57.8	37.4	88.7	50.0	41.9	89.3	60.1	55.2
Cys	10.0	7.6	8.9	7.0	6.2	20.6	11.0	7.6	17.2
Glu	14.2	67.4	60.5	91.0	79.7	82.0	130	78.2	91.2
Gly	33.4	17.9	13.6	31.2	16.9	29.3	37.0	11.6	23.0
Pro	30.5	8.8	9.9	23.4	7.7	32.9	26.4	9.9	21.9
Ser	25.5	17.8	30.0	23.3	22.0	40.1	33.6	20.0	39.0
Try	29.0	18.6	16.9	27.0	18.6	25.3	31.7	20.3	20.3
Protein(g/100g)	14.1	5.11	5.11	14.1	8.54	8.24	13.4	7.54	4.93

pre-school child (FAO/WHO/UNU, 1985). In Table VI, Lys was limiting in raw wheat with a value of 0.76 (76 %) and correction value of 100/76 (1.32); Met + Cys was limiting in steeped wheat with a value of 0.61 (61 %); and correction value of 100/61 (1.64); Lys was also limiting in the germinated sample and with similar data as in raw sample.

Table VII depicts the summary of the statistical analysis of results depicted in Tables I, II (pI only), III, IV, V and VI. Let us just take results from Table I to illustrate the information from other Tables. The correlation coefficient (r_{xy}) between raw/steeped (R/S) was 0.9608 which was significantly different since r_c = 0.9608 was greater than tabular r- value or r_t = 0.468 at n-2 (=16) and r=0.05. The degree of association for the R/S, i.e. $(r_{xy})^2$ was 0.92 which was very high. The linear regression (R c) showed that for every unit increase in the AA of raw sample, there was a corresponding increase of 0.23 in the steeped sample. The coefficient of alienation (C_A) was low in R/S with a value of 0.23 but with a corresponding high value of index of forecasting efficiency (IFE) of 72 %. Actually IFE normally gives a value in the reduction of the error of prediction of relationship. Thus a value of IFE of 72 % means the error of prediction was just 28 % showing that-both variables were associated or related with one another in a linear way. This type of reasoning goes down for the other members of Table VII.

Conclusion

In conclusion, it is seen in Table VIII that TEAA trend was raw > germinated > steeped and also the TNEAA trend was raw > germinated > steeped in *Triticum durum.* Despite these overall observations, it was also noted that germination technique improved the levels of His, Gly, Ser, Met and Phe as well as their sparing partners: Cys for Met and Tyr for Phe. The germination technique is therefore recommended for improved TEAA before *T. durum* is processed for food consumption or formulation. On the overall comparison with millet (Adeyeye 2009) and sorghum (Adeyeye 2008a) amino acid profiles (Table IX): raw wheat was better than all the amino acid levels in millet and 16/17 (94.1 %) in sorghum raw samples; steeped wheat was better than all the amino acid levels in millet and 8/17 (52.9 %) in sorghum steeped samples; germinated wheat was better than all the amino acid levels in millet and 12/17 (70.6 %) in sorghum germinated samples. It should however be noted that whilst germinated pearl millet (as well) had the highest TEAA, the steeped sample had the highest TEAA in the sorghum.

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