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Research Article

AMINO ACIDS COMPOSITIONS OF ROASTED COCOA, COCOA NIBS AND COCOA SHELL

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Abstract

An investigation into the amino acids composition of roasted cocoa seeds, cocoa nibs and cocoa shell was carried out. The amino acids analysis principle was based on ion-exchange chromatography. The total amino acids composition had a trend as: roasted cocoa > cocoa nibs> cocoa shell. The most concentrated amino acid in the three samples was Glu (5.50 – 12.7 g/100g).Lys was similar in value in both roasted cocoa and cocoa nibs (4.26 g/100g). The % TEAA was 47.1 – 49.9, TAA was 40.4 – 76.4 g/100g, % Cys in TSAA was 41.7 – 58.2, EAAI was 0.745 – 1.04, P-PER1/P-PER2 was 1.12 – 3.42/ 1.17 – 3.39, BV1/BV2 was 60.9 – 85.1/ 64.1 – 84.5 and pI was 2.36 - 4.39. Scores of the amino acids showed Met to be limiting in the three samples on whole hen's egg scores (0.23 -0.38), Lys was limiting on pre-school child (2-5 y) standards (0.40 – 0.73), while Met + Cys was limiting in roasted cocoa and cocoa nibs (0.52 – 0.59), it was Lys (0.42) in the shell when considered under the essential amino acid provisional amino acids scoring pattern. Results showed that shell would contribute reasonably to the amino acids of whole cocoa seed.

Keywords: Amino acids compositions, cocoa seed parts

Introduction

Cocoa belongs to the genus *Theobroma* in the family of the Sterculiaceae. Over 20 species of *Theobroma* are recognized. The cocoa cultivated for the international market belongs to the single species *Theobroma cacao* (L.). There are three large and distinct groups within the species of *T. cacao*. These are the Criollo, the Trinitario and the Forastero Amazonian (Opeke, 1992). The tree is called cacao while the bean is called cocoa.

The Forastero Amazonian group originated in the upper Amazon Basin, and it contains most of the cocoa commercially grown in Brazil, West Africa, Central America and Caribbean Islands. Selections were made in Trinidad from material collected by Pound in 1938 and 1943 when he was searching for the trees with resistance against *Marasmius perniciosus.* Posnette A.F. (1943) in search for resistance to the West Africa cacao viruses, introduced these Upper Amazon varieties to Tafo, Ghana (Opeke, 1992). The Forastero group is characterized by green pods, absence of anthocyanin pigmentation, thick pericarp, strongly lignifies mesocarp, plump but slightly flattened beans, deep purple cotyledons when fresh. The Forastero cacaos have done very well in West Africa mainly on account of their superior vigour of growth, precocity, mild to strong tolerance to West African virus strains and high bean yields.

With particular reference to Nigeria, cocoa is the most valuable agricultural export produce obtained in exportable quantities in Ondo, Ekiti, Osun, Oyo, Edo, Delta, Cross Rivers and Imo States. Cocoa was introduced into Nigeria in about 1894 by Squiss Ibaningo though commercial planting did not start until around

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1914. Nigeria is the world's fourth largest cocoa producer behind Ivory Coast, Ghana and Indonesia (ADM Cocoa, 2009). Nigeria's Production was 240,000 tonnes this season. Ivory Coast produced 1.73 million tonnes in the current season while Ghana produced 920,000 tonnes. The type of cacao grown in Nigeria is the Amelonado (melon shaped), a variety of Forastero.
The pods grow directly from the trunk of the tree. Mostly, they are harvested by hand using long-handled cutting tools and broken open to reveal the beans and the white pulp surrounding them. Beans are then extracted and are directly subjected to fermentation. The traditional process in West Africa, the world's largest growing area, is simple. Farmers place the pulp-covered beans on the ground, cover them with layers of leaves (often banana), and allow the heap to remain for 4 – 7 days, depending on the variety of the bean. It is preferable to mix the heap every two days so that the beans mix ferments evenly. The fermentation is critical for the future development of the colour and flavour of the cocoa, although there are still many unknowns as to the exact process occurring. Development of aroma precursors is essential to the eventual creation of flavours. During the process, change in colour occurs from the original light violet to dark brown. After drying in the sun, the beans are ready for shipment to the cocoa products manufacturers.

Cocoa products are eaten mainly because they are liked by young and old, owing to their attractive flavours and appearance which gives pleasure in eating (Minife, 1989). The nutritional parameters of cocoa are determined largely by the chemical composition of the material. The energy contributed to daily diet is dependent on the quantity of protein, carbohydrates and fats in the cocoa products and its corresponding digestibility coefficient (Minife, 1989). Cocoa powder is mainly used for low calorie food products. The minerals present in cocoa powder are important for their nutritional value. Olaofe et al. (1987) reported on the quality parameters of the cocoa beans from Nigeria, as well as on cocoa-based beverages of different brands consumed in the Nigerian market. The level of fermentation, degree of alkalization, roasting and fat content determine the colour and flavour of cocoa products. Fermentation helps to generate proper aroma and reduce the level of acetic acid, which causes off-flavour chocolate. The pH of cocoa liquors prepared from well fermented and dried West African beans is around 5.5 whilst those of unfermented or poorly fermented beans are 5.0 or less [The Biscuit, Cake, Chocolate and Confectionary Alliance (BCCCA), 1996]. Cocoa is added to cigarette for flavour enhancement. It also contains various psychoactive compounds, such as theobromine, caffeine, serotonin, histamine, tryptophan, trytamine, phenylethylamine, octapamine and anandamide (Rambali et al., 2002). The levels of those compounds in added cocoa in cigarette are thus critical to curtail possible addiction to cigarettes are thus critical to curtail possible addiction to cigarette smoking. Theobromine and theophylline, as well as caffeine, all found in this plant, are used as a diuretic, stimulant and also, in modern medicine, as an antiasthmatic (Morgan, 1994).

In addition to the work of Olaofe et al. (1987), Adeyeye et al. (2010) reported on the effect of farm and industrial processing on the amino acid profile of cocoa beans. Adeyeye and Ayejuyo (2011) evaluated the proximate and mineral compositions of cocoa nibs and shells of processed ungerminated and germinated cocoa beans.
There is, at present, scanty information on the amino acid profiles comparing roasted cocoa, its nibs and the shell from the industrially processed cocoa products on their relative concentrations. This study attempts to evaluate the amino acids composition of roasted cocoa seeds, their nibs and the cocoa bean coat (shell) from a major cocoa processing industry in Nigeria. The factory samples came from a blend of cocoa beans from different sources of the same species, which is the Forastero Amazonian Group. The first thing to understand is that cocoa is a mild acid. Regular cocoa powder is made from cocoa nibs: the inner kernel of the cocoa bean. These nibs have been pressed to remove part of the cocoa butter, leaving some cocoa fats and compressed cocoa particles. These are ground and the final product is a powder with a pH of about 5.5. This is the natural, unsweetened cocoa powder.

Materials and Methods

Materials

Samples of natural cocoa powder, cocoa nib and shell were collected from the production line of Ile-Oluji Cocoa Products Ltd, Ile-Oluji, Ondo State, Nigeria in November, 2011, for comparative analysis. The production process involves production of process line cocoa nibs (P-LCN) from cocoa beans. The process-line cocoa nibs (P-LCN) is prepared from blended, cleaned and destoned dried cocoa beans from the factory's cleaner/destoner machine. The processes involve microwaves heating of the beans at a temperature range of 90 - 100^0C for the period of about 15 minutes on a vibratory bed (this makes the cocoa bean shell puff for easy winnowing), automated roasting (at temperature range of 90 – 100^0C for about 20 minutes in a rotary evaporator). The factory samples were labeled roasted cocoa (A1), cocoa nibs (B1) and cocoa shell (C1) for the various analyses.

Sample treatment

The samples (A1, B1 and C1) were homogenised and ground to powder, using a Mouliness blender.

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The ground portions were kept in plastic bottles in the freezer (-4^{0}C) pending analysis.

Determination of amino acids

The amino acid profile in the cocoa samples was determined using methods described by Adeyeye et al. (2010). The cocoa samples were dried to constant weight. The mass was subsequently defatted, hydrolysed, filtered to remove the humins and evaporated to dryness at 40^{0}C under vacuum in a rotary evaporator. Each residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in a plastic specimen bottle kept inside the deep freezer pending subsequent analysis. The Technicon Sequential Multisample Amino Acid Analyser (TSM), Technicon Instruments Corporation, New York was used for the analysis. The principle is based on ion-exchange chromatography (IEC) (FAO/WHO, 1991). The equipment is designed to separate free acidic, neutral and basic acids of the hydrolysate. The amount loaded for each sample was 5-10µl and about 76 minutes elapsed for each analysis. The column flow rate was 0.50ml/min at 60^{o}C with reproducibility consistent within ±3%. The net height of each peak produced by the chart record of the TSM was measured and calculated for the amino acid it was representing. All chemicals used were of analytical grade.

Estimation of quality of dietary protein

Various methods were used for the above estimation.

I. The essential amino acid score was calculated using the provisional essential amino acid scoring pattern (FAO/WHO, 1973).
II. Amino acid score based on pre-school child essential amino acid requirement for ages 2 – 5y (FAO/WHO/UNU, 1985)
III. Amino acid score (for both essential and non-essential amino acids) was calculated based on whole hen's egg (Paul et al., 1976).
IV. Calculation of essential amino acid index (EAAI) (Nielsen, 2002).
V. Computation of protein efficiency ratio (C-PER or P-PER) was done using the equation suggested by Alsmeyer et al. (1974):
P-PER1 = -0.684 + 0.456(LEU) – 0.047(PRO)
P-PER2 = -0.468 + 0.454(LEU) – 0.105(TYR)
VI. Computation of biological value (BV) was calculated following the equation of Oser (1959) as follows:
    a. BV = 49.09 + 10.53 (PER)
    b. Where, PER = Protein Efficiency Ratio.
VII. The ratio of total essential amino acid (TEAA) to the total amino acid (TAA), i.e. (TEAA/TAA), total sulphur amino acid (TSAA), percentage cystine in TSAA (% Cys/TSAA), total aromatic amino acid (TArAA), total basic amino acid (TBAA), total acidic amino acid (TAAA), total neutral amino acid (TNAA) and the Leu/Ile ratios were calculated.

Other calculations completed were the determination of isoelectric point (pI) (Finar, 1975; Olaofe and Akintayo, 2000), grand mean, standard deviation and coefficients of variation in percentage. Also calculated were the various amino acid groups into classes I – VII (Nieman et al*.,* 1992) and summary of the amino acid profiles into Factors A and B means.

Results

In Table 1, the amino acids compositions of roasted cocoa (A1), cocoa nibs (B1) and cocoa shell (C1) were presented. The total amino acid (AA) had this pattern in g/100g: A1 (76.4) > B1 (72.9) > C1 (40.4). The inter-composition variation of the AA was generally with most values having coefficient of variation (CV %) being less than 50.0 except in Ala with CV % of 55.2. The least varied AA was Cys with CV % of 10.7. The most concentrated AA in the three samples was Glu having results of 5.50 – 12.7 g/100g, this was closely followed by another acidic AA, Asp with values of 4.53 - 9.15 g/100g. In all the AA determined, C1 was the consistently lowest on pair-wise comparison except in Cys where C1 (0.93 g/100g) > A1 (0.86 g/100g). The most concentrated essential AA (EAA) was Leu with value range of 4.10 – 9.30 g/100g protein and closely followed by Phe with values of 2.65 – 4.46g/100g protein.

Table 2 contained the summary of the differences between amino acid profiles of the roasted cocoa and cocoa nibs (A1 – B1) and roasted cocoa and cocoa shell (A1 – C1). The differential variations had very high values with CV % of 3.07 – 778. Equivalent values of AA were recorded in A1 and B1 in Gly as well as in Lys, hence A1 – B1 in each case was 0.00 (0.00%). Positive differences were recorded in favour of A1 in Ser, Pro, Thr, Ile, Met, Asp, Glu, Arg and total AA whereas it was positive for B1 in Ala, Val, Leu, Phe, His, Tyr and Cys, that is 9/7 in favour of A1 against B1. In A1 – C1, all the parameters were positive for A1 except in Cys which was positive for C1, that is 17/1 in favour of A1 against C1.

The quality parameters of the amino acids profiles of the samples could be seen in Table 3. All the CV % values were less than 50 except in Leu-Ile with a value of 54.0 %. The TEAA had a range of 20.2 – 36.0 g/100g with corresponding % TEAA range of 47.1 – 49.9. the TNEAA range was 20.3 – 40.3g/100g protein with corresponding % TNEAA range of 50.1 – 52.9. The TSAA was low at

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Table 1. Amino acids compositions of roasted cocoa, cocoa nibs and cocoa shell (g/100g)

Amino acids	A1	B1	C1	Mean	SD	CV%
Glycine	4.10	4.10	2.06	3.42	1.18	34.4
Alanine	3.30	3.86	1.02	2.73	1.50	55.2
Serine	3.50	2.80	2.06	2.79	0.72	25.8
Proline	3.70	2.84	1.32	2.62	1.21	46.0
Valine	3.15	3.80	2.70	3.22	0.55	17.2
Threonine	3.40	3.30	1.85	2.85	0.87	30.4
Isoleucine	3.23	2.85	2.30	2.79	0.47	16.7
Leucine	8.16	9.30	4.10	7.19	2.73	38.0
Methionine	1.20	0.75	0.73	0.89	0.27	29.8
Aspatic acid	9.15	7.66	4.53	7.11	2.36	33.1
Lysine	4.26	4.26	2.30	3.61	1.13	31.4
Glutamic acid	12.7	11.4	5.50	9.84	3.81	38.7
Phenylalanine	4.37	4.46	2.65	3.83	1.02	26.7
Histidine	2.55	2.61	1.26	2.14	0.76	35.6
Arginine	5.69	4.40	3.02	4.37	1.34	30.6
Tyrosine	3.17	3.50	2.10	2.92	0.73	25.0
Cystine	0.86	1.06	0.93	0.95	0.10	10.7
Total	76.4	72.9	40.4	63.3	19.8	31.4

A1 = roasted cocoa, B1= cocoa nib, C1 = cocoa shell, SD = standard deviation, CV% = coefficient of variation.

Table 2. Summary of the differences between amino acid profiles of the roasted cocoa and cocoa nibs (A1-B1) and roasted cocoa and cocoa shell (A1-C1)

Amino acids	A1-B1 (%)	A1-C1 (%)	Mean	SD	CV%
Glycine	0.00(0.00)	+2.04(+49.8)	1.02	1.44	141
Alanine	-0.56(-17.0)	+2.28(+69.1)	0.86	2.01	234
Serine	+0.70(+20.0)	+1.44(+41.1)	1.07	0.52	48.9
Proline	+0.86(+23.2)	+2.38(+64.3)	1.62	1.07	66.3
Valine	-0.65(-20.6)	+0.45(+14.3)	-0.10	0.78	778
Threonine	+0.10(+2.94)	+1.55(+45.6)	0.83	1.03	124
Isoleucine	+0.38(+11.8)	+0.93(+28.8)	0.66	0.39	59.4
Leucine	-1.14(-14.0)	+4.06(+49.8)	1.46	3.68	252
Methionine	+0.45(+37.5)	+0.47(+39.2)	0.46	0.01	3.07
Aspatic acid	+1.49(+16.3)	+4.62(+50.5)	3.06	2.21	72.4
Lysine	0.00(0.00)	+1.96(+46.0)	0.98	1.39	141
Glutamic acid	+1.29(+10.2)	+7.15(+56.5)	4.22	4.14	98.2
Phenylalanine	-0.09(-2.06)	+1.72(+39.4)	0.82	1.28	157
Histidine	-0.06(-2.35)	+1.29(+50.6)	0.62	0.95	155
Arginine	+1.29(+22.7)	+2.67(+46.9)	1.98	0.98	49.3
Tyrosine	-0.33(-10.4)	+1.07(+33.8)	0.37	0.99	268
Cystine	-0.20(-23.3)	-0.07(-8.14)	-0.14	0.09	68.1
Total	+3.53(+4.62)	+36.01(+47.1)	19.8	23.0	116

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Table 3. Quality parameters of the amino acids profiles of roasted cocoa, cocoa nibs and cocoa shell

Parameter	A1	B1	C1	Mean	SD	CV%
TAA	76.4	72.9	40.4	63.3	19.9	31.4
TEAA with His	36.0	35.7	20.2	30.6	9.06	29.6
TEAA without His	33.5	33.1	18.9	28.5	8.30	29.1
TNEAA	40.3	37.2	20.3	32.6	10.8	33.1
TArAA	7.54	7.96	4.75	6.75	1.74	25.8
TAAA	21.8	19.0	10.0	17.0	6.15	36.3
TBAA	12.5	11.3	6.58	10.1	3.12	30.9
TNAA	6.90	6.10	3.91	5.64	1.55	27.5
TSAA	2.06	1.82	1.66	1.85	0.20	10.9
%TEAA with His	47.1	49.0	49.9	48.7	1.43	2.94
%TEAA without His	43.8	45.4	46.8	45.3	1.50	3.31
%TNEAA	52.9	51.0	50.1	51.3	1.43	2.78
%TArAA	9.86	10.9	11.8	10.8	0.95	8.74
%TAAA	28.5	26.1	24.8	26.5	1.88	7.09
%TBAA	16.4	15.5	16.3	16.1	0.49	3.07
%TNAA	9.03	8.37	9.67	9.02	0.65	7.20
%TSAA	2.69	2.50	4.11	3.10	0.88	28.4
Cys in TSAA	0.86	1.06	0.93	0.95	0.10	10.7
% Cys in TSAA	41.7	58.2	56.0	52.0	8.96	17.2
P-PER 1	2.86	3.42	1.12	2.47	1.20	48.6
P-PER 2	2.90	3.39	1.17	2.49	1.17	46.9
Leu/Ile	2.53	3.26	1.78	2.52	0.74	29.3
Leu-Ile	4.93	6.45	1.80	4.39	2.37	54.0
%Leu-Ile	60.4	69.5	43.9	57.9	13.0	22.4
EAAI	1.03	1.04	0.75	0.94	0.17	17.9
pI	4.39	4.20	2.36	3.65	1.12	30.7
BV 1	79.2	85.1	60.9	75.1	12.6	16.8
BV2	79.6	84.5	61.4	75.2	12.2	16.2

TAA = total amino acids, TAAA = total acidic amino acids, TEAA = total essential amino acids, TNEAA = total non-essential amino acids, TArAA= total aromatic amino acids, TSAA = total sulphur amino acids, TBAA= total basic amino acids, TNAA = total neutral amino acids, pI = iso-electric point, P-PER1 = predicted protein efficiency ratio 1 (with respect to Leu and Pro), P-PER1 = predicted protein efficiency ratio 2 (with respect to Leu and Tyr), BV 1 = Biological value (with respect to P-PER1), BV2 = Biological value (with respect to P-PER2), EAAI = essential amino acids index

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1.66 – 2.06 g/100g with % TSAA of 2.50 – 4.11. The P-PER1 and P-PER2 had close values as P-PER1 (1.12 – 3.42) and P-PER2 (1.17 – 3.39); this was followed in virtually similar pattern by BV1 and BV2 as BV1 (60.9 – 85.1) and BV2 (61.4 – 84.5). The ratio of Leu/Ile was low at 1.78 – 3.26 but % Leu- Ile was high at 43.9 – 69.5. The EAAI was typical of most plants at 0.745 – 1.04 whereas the pI showed that the samples were acidic at pI values of 2.36 – 4.39.

In Table 4, the amino acids scores of the samples based on whole hen's egg were shown. In A1, these AA had scores > 1.0: Gly, Glu and His; in B1, they were: Gly, Leu and His but none in C1. The limiting amino acid (LAA) in A1 was Met (0.38), B1 was Met (0.23) and in C1, it was Ala (0.19). The CV % was not high in most cases of the AA. In Table 5, the EAA scores (EAAS) based on the provisional scoring pattern were depicted. In A1, EAAS was greater than 1.0 in Leu and Phe +Tyr with LAA of 0.59 in Met + Cys; in B1, EAAS was greater than 1.0 in Leu and Phe+Tyr with LAA of 0.52 in Met + Cys; in C1 all EAAS values were less than 1.0 but LAA was Lys with a value of 0.42. The highest CV % here was 38.0 recorded for Leu. Table 6 contained the EAAS of the samples based on the requirements of pre-school child (2-5 y). In A1, EAAS > 1.0 were Leu, Ile, Phe + Tyr, Thr, His and total EAA; in B1, EAAS > 1.0 were Leu, Ile, Phe +Tyr, Val, His and TEAA whereas all EAAS in C1 were < 1.0. The LAA values were 0.73 (Lys in A1), 0.73 (Lys in B1) and 0.40 (Lys in C1). The CV % was generally low at 10.9 – 38.0.

The amino acid groups into classes I to VII was shown in Table 7. The general trend in class concentration of AA could be seen as follows: Class I > IV > V > VI > II> VII >III. This meant that the most concentrated AA was AA with aliphatic side chains whereas the least concentrated was AA with side chains containing sulphur atoms. The CV % values were also generally low at 11.0 – 46.0. The summary of the amino acid profiles into factors A and B means was depicted in Table 8. The TEAA and TNEAA mean values were close at 30.6 – 32.6 g/100g protein with Factor A and B means having a value of 31.6 g/100g protein.

Discussion

In the total amino acid profiles based on pairwise comparison, the following AA were most concentrated in A1 than the other two samples: Ser, Pro, Thr, Ile, Met, Asp, Glu, Arg or 9/17 (52.9 %); in B1, the following AA were mostly concentrated: Ala, Val, Leu, Phe, His, Tyr, Cys or 7/17 (41.2%).Two AA were of equivalent levels in both A1 and B1: Lys and Gly or 2/17 (11.8%). Whereas all the AA values in B1 were correspondingly greater than in C1, not all AA values in A1 were greater than the corresponding AA in C1, in fact, Cys in C1 (0.93g/100g) was greater than in A1 (0.86g/100g). One AA was 1/17 (5.88%). It would appear that the presence of shell might not be good enough to predict the value of AA in roasted natural cocoa vis-à-vis the cocoa nibs, or else, A1 would have been expected to be much higher than B1 in the following amino acids: Gly, Ala, Val, Leu, Lys, Phe, His and Tyr. This could be due to the little weight contributed to cocoa beans by the shell. Both A1 and B1 shared equally, the levels of EAA as follows: in A1 we had Thr, Ile, Met and Arg; in B1 we had Val, Leu, Phe and His; equivalent level in A1 and B1 was recorded for Lys.

The crude protein compositions of the samples were (g/100g): 22.8 (A1), 22.3 (B1), 13.1 (C1) which had been reflected in their various total AA levels. The protein levels were generally low. The low level of crude protein in the samples could have been due to the Maillard reactions which are an interaction between the carbonyl group of a reducing sugar and the free amino acid group from an amino acid or protein. The resulting condensation product is converted by the Amadori rearrangement to the 1-deoxy-2-ketosyl compound. Browning then proceeds along complex pathways, the exact sequence being dependent on pH, temperature, concentration and the identity of the reactants (Muller and Tobin, 1980). From these results, both samples A1 and B1 would be the best protein food ingredient than sample C1.

The summary of the differences between the amino acids of A1 – B1 and A1-C1 made the sample A1 as constant whereas B1 and C1 were the variables, where + sign appeared before a value, it meant that A1 was higher than either B1 or C1, where – sign preceded a value, it meant A1 was lower than B1 and where 0.00 (0.00%) was recorded, it meant that both A1 and B1 had equivalent values. This summary further explained the observations in Table 1.

The quality parameters showed the TAA to vary from 40.4 to 76.4 g/100g protein. The TAA in A1 and B1 are close to the value of 65.9 g/100g cp in *Anacardium occidentale* whereas TAA in C1 is close to the value of 35.6 g/100g cp in *Cola acuminata* (Adeyeye et al*.,* 2007). The TAA in A1 and B1 are close to the value of 64.1 g/100g cp in unfermented cocoa nibs, whilst C1 TAA is close to the value of 36.8 g/100g cp in processed cocoa cake samples (Adeyeye et al*.,* 2010). Our samples TNEAA range was 20.3 – 40.3 g/100g; the TNEAA in B1 and A1 (37.2 – 40.3g/100g) are close to the value range of 32.7 – 45.5 g/100g cp in African yam bean (Adeyeye, 1997) and also close to 32.3 g/100g cp in *A. occidetale* (Adeyeye et al*.,* 2007). The TEAA with His had this pattern (g/100g):

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Table 4. Amino acids scores of roasted cocoa, cocoa nibs and cocoa shell based on whole hen's amino acids scoring pattern (Paul *et al.,*1976)

Amino acids	A1	B1	C1	Mean	SD	CV%
Glycine	1.37	1.37	0.69	1.14	0.39	34.4
Alanine	0.61	0.71	0.19	0.50	0.28	55.2
Serine	0.44	0.35	0.26	0.35	0.09	25.8
Proline	0.97	0.75	0.35	0.69	0.32	46.0
Valine	0.42	0.51	0.36	0.43	0.07	17.2
Threonine	0.67	0.65	0.36	0.56	0.17	30.4
Isoleucine	0.58	0.51	0.41	0.50	0.08	16.7
Leucine	0.98	1.12	0.49	0.87	0.33	38.0
Methionine	0.38	0.23	0.23	0.28	0.08	29.8
Aspatic acid	0.86	0.72	0.42	0.66	0.22	33.1
Lysine	0.69	0.69	0.37	0.58	0.18	31.4
Glutamic acid	1.05	0.95	0.46	0.82	0.32	38.7
Phenylalanine	0.86	0.87	0.52	0.75	0.20	26.7
Histidine	1.06	1.09	0.53	0.89	0.32	35.6
Arginine	0.93	0.72	0.50	0.72	0.22	30.6
Tyrosine	0.79	0.88	0.53	0.73	0.18	25.0
Cystine	0.48	0.59	0.52	0.53	0.06	10.7
Total	0.78	0.74	0.41	0.64	0.20	31.4

Table 5. Amino acids scores of roasted cocoa (A1), cocoa nibs (B1) and cocoa shell (C1) based on provisional amino acids scoring pattern (FAO/WHO, 1973)

Amino acids	A1	B1	C1	Mean	SD	CV%
Leucine	1.17	1.33	0.59	1.03	0.39	38.0
Isoleucine	0.81	0.71	0.58	0.70	0.12	16.7
Lysine	0.77	0.77	0.42	0.66	0.21	31.4
Methionine + Cystine	0.59	0.52	0.47	0.53	0.06	10.9
Phenylalanine + Tyrosine	1.26	1.33	0.79	1.13	0.29	25.8
Threonine	0.85	0.83	0.46	0.71	0.22	30.4
Valine	0.63	0.76	0.54	0.64	0.11	17.2
Total	0.91	0.95	0.56	0.81	0.21	26.5

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Table 6. Amino acids scores of roasted cocoa (A1), cocoa nibs (B1) and cocoa shell (C1) based on pre-school child (2-5 yrs) amino acids scoring pattern (FAO/WHO/UNU, 1985)

Amino acid	A1	B1	C1	Mean	SD	CV%
Leucine	1.24	1.41	0.62	1.09	0.41	38.0
Isoleucine	1.15	1.02	0.82	1.00	0.17	16.7
Lysine	0.73	0.73	0.40	0.62	0.20	31.4
Methionine + Cystine	0.82	0.73	0.66	0.74	0.08	10.9
Phenylalanine + Tyrosine	1.20	1.26	0.75	1.07	0.28	25.8
Threonine	1.00	0.97	0.54	0.84	0.26	30.4
Valine	0.90	1.09	0.77	0.92	0.16	17.2
Histidine	1.34	1.37	0.66	1.13	0.40	35.6
Total	1.05	1.09	0.64	0.93	0.25	27.1

Table 7. Amino acid groups of roasted cocoa, cocoa nibs and cocoa shell (g/100g)

Class	A1	B1	C1	Mean	SD	CV%
I	21.9	23.9	12.2	19.3	6.28	32.5
II	6.90	6.10	3.91	5.64	1.55	27.5
III	2.06	1.81	1.66	1.84	0.20	11.0
IV	21.9	19.1	10.0	17.0	6.18	36.4
V	12.5	11.3	6.58	10.1	3.12	30.9
VI	10.1	10.6	6.01	8.89	2.51	28.2
VII	3.70	2.84	1.32	2.62	1.21	46.0

A1= roasted cocoa, B1= cocoa nib, C1= cocoa shell,
Amino acid groups:
CLASS I= with aliphatic side chains (hydrogen and carbons), II= with side chains containing hydroxylic (OH) groups, III= with side chains containing sulphur atoms, IV= with side chains containing acidic groups or their amides, V= with side chains containing basic groups, VI= with side chains containing aromatic rings, VII= imino acids

Table 8. Summary of the amino acid profiles into factors A and B

Amino Acid composition (Factor B)	Samples (Factor A)			Factor B means
	A1	B1	C1	
Total Essential Amino Acid	36.0	35.7	20.2	30.6
Total Non-Essential Amino Acid	40.3	37.2	20.3	32.6
Factor A means	38.2	36.5	20.2	31.6

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A1 (36.0) > B1 (35.7) > C1 (20.2). However, on percentage basis, there was a reversal in the pattern as: C1 (49.9 %) > B1 (49.0 %) > A1 (47.1 %). The CV % of %TEAA was low at 2.94. The TNEAA of 20.3 - 40.3 g/100g had similar pattern of sample range in the % TNEAA with values of 50.1 – 52.9 with a CV % of 2.78. The CV % of TEAA and TNEAA were close at 29.1 – 33.1. The TNAA range was 3.91 – 6.90 g/100g with 27.5 % CV; the % TNAA values were much closer with values of 8.37 – 9.67 and CV % of 7.20.

The % TEAA of 47.1 – 49.9 were all above the 39 % considered adequate for ideal protein food for infants, 26 % for children and 11 % for adults (FAO/WHO, 1973). The contents of TSAA (1.66 – 2.06 g/100g) were generally lower than 5.80 g/100g recommended for infants (FAO/WHO, 1973). The TArAA range suggested for ideal infant protein is 6.8 – 11.8 g/100g; our results in A1 (7.54 g/100g) and B1 (7.96 g/100g) were within this range whereas C1 (4.75 g/100g) is below this range. The TAAA values of 10.0 – 21.8 g/100g were much higher than the TBAA in all the samples with values of 6.58 – 12.5 g/100g with respective values of CV % as 36.3 and 30.9.

The TEAA in melon and gourd oil seeds with respective values of 534 mg/g cp and 536mg/g cp (Olaofe et al., 1994) are higher than the present results that ranged from 202-360 mg/g cp; all of our EAA values are lower than those in soybean (444g/100g cp) (Kuri et al., 1991). Our present samples are either close to or lower than the following TEAA levels (mg/100g cp): pigeon pea (452) (Nwokolo, 1987), pumpkin seed (396) (Aisegbu, 1987), cowpea (426) (Olaofe et al., 1993) and *Cajanus cajan* (436) (Oshodi et al., 1992). This meant that our samples were of lower protein quality than those of cowpea, soybean, pigeon pea and pumpkin seed. However, whilst Cys was 0.0 mg/g cp in melon, pumpkin seed and gourd seed (Olaofe et al., 1994) and 11.3mg/g cp in *A. occidentale*, 2.5mg/gcp in *G. kola* and 4.5mg/g cp in *C. acuminata* (Adeyeye et al., 2007), it is (g/100g cp) in: Al (0.86), Bl (1.06) and Cl (0.93). Generally, most of our results are better in many of the amino acids (essential and non-essential) than is in pumpkin seed (Olaofe et al., 1994).

Whilst it is known that cystine can spare part of the requirement of methionine, FAO/WHO/UNU (1985) does not give any indication of the proportion of total sulphur amino acids that can be met by Cys. For rat, chick and pig, the proportion is about 50% (FAO/WHO, 1991). Most animal proteins are low in cystine; in contrast, many vegetable proteins, especially the legumes, contain substantially more Cys than methionine. Thus, for animal protein, Cys is unlikely to contribute more than 50% of the total sulphur amino acids (FAO/WHO, 1991). For our samples, the percentages of Cys in total sulphur amino acids were: 41.7 (Al), 58.2 (Bl) and 56.0 (Cl), with Al behaving like an animal protein whereas Bl and Cl behaving like plant proteins. The (Cys/TSAA)% values in samples Al and Bl are higher than the values in unfermented nibs (44.1%), fermented nibs (46.3%) (Adeyeye et al., 2010). The values of 56.0 (Cl) and 58.2 (Bl) are close to the value of 50.5% in *A. occidentale* (Adeyeye et al., 2007), 62.9% (coconut endosperm) (Adeyeye, 2004) and 58.9% (raw guinea corn) (Adeyeye, 2008). FAO/WHO (1973), states that Cys may supply up to one-third of the need for total sulphur amino acids whilst tyrosine may also supply up to one-third of the need for total aromatic amino acids.

The predicted protein efficiency ratios (P-PER) were 2.86/2.90 (Al), 3.42/3.39 (Bl) and 1.12/1.17 (Cl). All these values are lower than 3.55 (unfermented cocoa) but higher than 2.55 (fermented cocoa) nibs in Al and Bl; also higher than in the P-LCN (2.47) and PCCS (2.02) samples (Adeyeye et al., 2010). 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). These results show that cocoa shell (Cl) may likely be less utilized in the body than would the other cited literature samples.

The Leu/Ile ratio values ranged as follows: 2.53 (Al), 3.26 (Bl) and 1.78 (Cl). From Table 1, the levels of Leu/Ile are 8.16/3.23 (2.53, Al), 9.30/2.85 (3.26, Bl) and 4.10/2.30 (1.78, Cl) showing Leu to be about 2½ parts of Ile in Al, about 3 parts of Ile in Bl and above 1½ parts of Ile in Cl. The ideal value of Leu/Ile is 2.36 (FAO/WHO, 1991). It has been suggested that an amino acid imbalance from excess leucine might be a factor in the development of pellagra in sorghum consumption (FAO, 1995). Deosthale et al. (1970) showed that excess leucine in foods interfered with the utilization of isoleucine and lysine.

High Leu in the diet impairs tryptophan and niacin metabolism and is responsible for niacin deficiency in sorghum eaters (FAO, 1995). This leads to the hypothesis that excess Leu in sorghum is aetiologically related to pellagra in sorghum-eating populations (FAO, 1995). A study (FAO, 1995), had suggested that Leu/Ile balance is more important than dietary excess of Leu alone in regulating the metabolism of Trp and niacin and hence the disease process. Experiments in dogs have shown that animals fed sorghum proteins (with less than 110mg/g cp of Leu) did not suffer from nicotinic acid deficiency. It is gratifying to note that none of our samples have levels of Leu up to 110mg/g cp.

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The essential amino acid index (EAAI) ranged from 0.745 to 1.04 with CV% (17.9). EAAI is useful as rapid tool to evaluate food formulations for protein quality, although it does not account for difference in protein quality due to various processing methods or certain chemical reactions (Nielsen, 2002). The EAAI of defatted soybean is 1.26 (Nielsen, 2002). The Biological Value (BV) values ranged as follows: BV1/BV2 60.9-85.1/61.4-84.5. BV is a scale of measurement used to determine what percentage of a given nutrient source is utilized by the body. The theoretical highest BV of any food source is 100%. In short-BV refers to how well and how quickly your body can actually use the protein you consume (food-info.net/uk/protein/bvi/htm). The BV is very important for vegetarians and vegans, who do not consume animal protein. Plant proteins generally have lower content of some EAA such as Lys and Met. Some literature Biological value of some foods can be seen below: whole egg (93.7), milk (84.5) (similar to BV2 in Bl), fish (76.0), beef (74.3), soybeans (72.8), rice, polished (64.0), wheat, whole (64.0), corn (60.0) and beans, dry (58.0) (food-info.net/uk/protein/bv.htm). Our results in Al and Bl were better than many of the literature values. All the values for the isoelectric point (pI) were low at 2.36-4.39. During experiments on food functionality, one of the important parameters usually studied is the protein solubility of the sample. From such works the minimum solubility is normally observed. The information on minimum protein solubility is important in the preparation of protein isolates; thus, the calculation of pI from the amino acids would give a rough estimate of the pH to prepare the protein isolate of an organic substance without necessarily going through protein solubility determination.

In the amino acid scores based on whole hen's egg, Met was limiting in Al and Bl but Ala was limiting in Cl. Therefore, in order to fulfill the day's needs for the AA in the samples each, 100/38 or 2.63 times as much Al would have to be eaten when it is the sole protein source in the diet; in Bl, it would be 100/23 or 4.35 times the protein level; in C1, it would be 100/19 or 5.26 times the protein level. In the EAA scores based on FAO/WHO (1973) where TSAA was limiting in Al and Bl and Lys limiting in Cl, the corrections would be 100/59 or 1.69 times in Al, 100/52 or 1.92 times in Bl and 100/42 or 2.38 times in Cl. On scores based on pre-school child EAA requirement (FAO/WHO/UNU, 1985), Lys was limiting in Al and Bl with similar value of 0.73, hence similar correction values would be needed for Al and Bl and 100/73 or 1.37 times whereas Lys also limiting in Cl had a value of 0.40 and correction factor of 100/40 or 2.50 times. In Table 6, scores for Lys and TSAA are similar with a value of 0.73. However, Lys is limiting because in hierarchy of AA limiting arrangement, first LAA acid is Lys, second is TSAA, third is Thr and fourth is Trp (Bingham, 1977).

In the amino acids (AA) classification into groups, the followings were observed (Nieman et al., 1992). Class I [AA with aliphatic side chains (hydrogen and carbons)] are Gly, Ala, Val, Leu and Ile with total AA values of 21.9g/100g cp (Al) < 23.9g/100g cp (Bl) > 12.2g/100g cp (Cl). Class II [AA with side chains containing hydroxylic (OH) groups] are Ser, Thr with total values of 6.90g/100g cp (Al) > 6.10g/100g cp (Bl) > 3.91g/100g cp (Cl). Class III [AA with side chains containing sulphur atoms] are Cys, Met with total values of 2.06g/100g cp (Al) > 1.81g/100g cp (Bl) > 1.66g/100g cp (Cl). Class IV [AA which side chains containing acidic groups or their amides] are Asp, Glu with total values of 21.9g/100g cp (Al) > 19.1g/100g cp (Bl) > 10.0g/100g cp (Cl). Class V [AA with side chains containing basic groups] are Arg, Lys, His with total values of 12.5g/100g cp (Al) > 11.3g/100g cp (Bl) > 6.58g/100g cp. Class VI [AA with side chains containing aromatic rings] are His, Phe, Tyr with total values of 10.1g/100g cp (Al) < 10.6g/100g cp (Bl) > 6.01g/100g cp (Cl). Class VII [imino acid] is Pro with values of 3.70g/100g cp (Al) > 2.84g/100g cp (Bl) > 1.32g/100g cp (Cl). The roasted cocoa (Al) had the highest concentration in classes II, III, IV, V, VI and VII (i.e. 6/7 or 85.7%) whilst cocoa nibs (Bl) was mostly concentrated in class I (i.e. 1/7 or 14.3%). Out of the nine EAA determined, 4/9 (i.e. 44.4%) were in Al groups of classes I, II, III and V whilst 4/9 (i.e. 44.4%) were in Bl groups of classes I, V and VI whereas Lys (similar in values in Al and Bl) formed 1/9 (i..e 11.1%) in group of class V.

The amino acid profiles (g/100g cp) for the samples as distributed into the TEAA and TNEAA and summarized into Factors A and B means gave a terminal value of 31.6 which is close to the Factor B means of 30.6 (TEAA) and 32.6 (TNEAA).

In conclusion, the finding of the study showed that there was more positive build up of AA in roasted cocoa than in the cocoa nibs and cocoa shell. However, the distribution and concentration of EAA appeared to be shared 50/50% in Al and Bl. The results of the AA profiles did not show appreciable contribution from shell to the roasted cocoa. Finally, the use of the samples for complementation/fortification would be determined by what the researcher wants to achieve; if it is for general protein improvement it would be Al > Bl > Cl but if it is complementation/fortification of EAA, the trend would be Al for Thr, Ile, Met and Arg; for Bl, it would be Val, Leu, Phe and His but Lys would be for both Al and Bl.

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