Food Chemistry 118 (2010) 357–363

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of farm and industrial processing on the amino acid profile of cocoa beans

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ARTICLE INFO

Article history:
Received 19 July 2008
Received in revised form 26 March 2009
Accepted 30 April 2009

Keywords:
Cocoa beans
Farm fermentation
Industrial processing
Amino acids

ABSTRACT

An investigation into the amino acid profiles of unfermented and fermented cocoa nibs, as well as process-line cocoa nibs (P-LCN) and processed cocoa cake samples (PCCS) was carried out. In the unfermented cocoa nibs, Glu (128 mg/g crude protein i.e. 128 mg/gcp) was the most abundant amino acid whilst the most concentrated essential amino acid in the same sample was Leu (72.2 mg/g crude protein); in the fermented cocoa nibs, a similar trend was observed, with respective values of Glu (153 mg/gcp) and Leu (62.4 mg/gcp). Lys (181 mg/gcp) was most abundant amino acid in P-LCN and Ile (63.3 mg/gcp) was the second most abundant; also, in PCCS, Lys (52.7 mg/gcp) was the most abundant amino acid but Asp (43.7 mg/gcp) was the second most abundant. The total amino acid content was (mg/gcp), 641 (unfermented nibs), 708 (fermented nibs), 635 (P-LCN) and 368 (PCCS), with corresponding essential amino acids of 300, 287, 478 and 185 (all with His), respectively. Based on whole hen's egg, the limiting amino acids for the samples were: Ser (unfermented nibs), Met (fermented nibs), Ala (P-LCN) and Val (PCCS), whereas under provisional amino acid scoring pattern, they were: Met + Cys (unfermented nibs), Met + Cys (fermented nibs), Thr (P-LCN) and Val (PCCS). Prolonged and high heat treatments appeared to have reduced the essential amino acids of the PCCS as compared to the P-LCN. Significant differences existed between contents of essential amino acids and non-essential amino acids at $p < 0.05$ in unfermented cocoa nibs, fermented cocoa nibs and P-LCN.

1. Introduction

Cocoa bean was the foremost Nigerian foreign exchange earner before the advent of crude oil and it is currently in the second position after petroleum. The annual production in Nigeria is about 165,000 metric tonnes (MT) (Akinyeye, 1999). A small percentage of the annual production is processed, locally, to cocoa butter and cocoa cake, whilst the bulk is exported. The cocoa beans of commerce are the seeds of the tree –*Theobroma cacao* (Linnaeus), properly harvested, fermented and dried. It originated from Latin America about 500 years ago and thence to Europe, from where it was introduced to other parts of the world (International Cocoa and Commodities Organisation, 2000). Cocoa bean is an oil seed, just like palm kernel, groundnut, sesame seed or any of the other oilseeds. However, it is rarely processed in the same way as the other oil seeds in order to get the oil. The reason for this is probably due to the unique physicochemical characteristics of the beans and its constituents, especially the fat. The Aztecs (of Mexico) prepared the first cocoa drink called "Chocolatl" about 500 years ago (Minife, 1989). This chocolate drink was prepared from a mixture of ground, roasted whole beans or "nibs" and sugar. The drink was extremely rich because of the high fat content. The richness of the beverage made the Aztecs believe that the cocoa tree was of divine origin, hence the name – "Theobroma" meaning, *food for the gods.* The drink has aphrodisiac properties and was held in high esteem as a nuptial aid.

To obtain cocoa, the harvested pods are fermented, by naturally occurring bacteria and yeasts to eliminate their natural, bitter, astringent quality, during which the seeds are cured and roasted. The clean kernels obtained after the removal of the shell, called cocoa nibs, are manufactured into various products. The larger percentage of the nibs is fat, removed by pressure, and is called cocoa butter which is used in fine soaps and cosmetics and in medicine for emollients and suppositories. The residue is ground to a powder (cocoa) and used for beverages and flavouring. Chocolate is a product in which the cocoa butter has been retained. Cocoa products have a high food value because of the large proportion of fat, carbohydrates, and protein. Cacao is classified in the division Magnoliophyta, class Magnoliopsida, order Malvales and family Sterculiaceae.

Cocoa products are eaten mainly because they are liked, by young and old, owing to their attractive flavours and appearance which give pleasure in eating (Minife, 1989). The nutritional parameters of cocoa are determined largely by the chemical composition of the material. The energy contribution to daily diets is dependent on the quantum of proteins, carbohydrates and fats in the cocoa product 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

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doi:10.1016/j.foodchem.2009.04.127

for their nutritional value. Olaofe, Oladeji, and Ayodeji (1987) and Olaofe and Onajeta (1986) reported on the quality parameters of 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 alkalisation, 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 in 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 (BCCCCA), 1996). Cocoa is added to cigarettes for flavour enhancement. It also contains various psychoactive compounds, such as theobromine, caffeine, serotonin, histamine, tryptophan, trytamine, tyramine, phenylethylamine, octopamine and anandamide (Rambali et al., 2002). The levels of these compounds in added cocoa in 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).

There is, at present, scanty information on the amino acid profile of cocoa beans and the effect of farm and industrial processing on their relative concentrations. This study attempts to evaluate the amino acid composition of dried unfermented and fermented cocoa beans from a major cocoa-producing town in south-west Nigeria vis-à-vis that of the in-process cocoa nibs and processed cocoa cake from a major cocoa processing industry in Nigeria. The effects of fermentation and heat treatment during the course of milling are to be evaluated. Direct processing of the same batch of beans collected from the selected farm location could not be conducted because of the enormous quantity required for batch processing. Consequently, heterogeneous samples of beans supplied, from different locations (thoroughly mixed together to a representative sample), were used for evaluating the effect of heat treatment during milling. This means the analyses were on four samples in two major groups; they are fermented and unfermented samples from the same farm in Aisegba Ekiti and this is the Forastero Amazonian Group (Opeke, 1992); the second group is the factory sample group consisting of the process-line cocoa nibs (P-LCN) and the processed cocoa cake samples (PCCS). The factory samples (P-LCN and PCCS) came from a blend of cocoa beans from different sources of the same species, which is the Forastero Amazonian Group.

2. Materials and methods

2.1. Materials

Cocoa bean seeds were collected from fully ripe pods harvested from some cocoa trees in a farm plantation located at Aisegba Ekiti in Ekiti State of Nigeria in December 2007, during the main crop season. The harvested beans were divided into two portions immediately after they were taken out of the pods. Whilst the first portion was directly sun-dried in open air, the second portion was fermented by a heaping method, using plantain leaves to cover the beans for 6 days before sun-drying. The dried beans were deshelled, dry-milled and labelled as unfermented and fermented nibs, respectively. These formed the group one samples. Similarly, in-process cocoa beans (heterogeneous) of indefinite source, and kibbled cake were collected from the production floor of Co-operative Cocoa Products Limited, Akure, Ondo State, Nigeria in December 2007, for comparative analysis.

The process-line cocoa nibs (P-LCN) were prepared from blended, cleaned and destoned dried cocoa beans from the factory's cleaner/destoner machine. The processed cocoa cake sample (PCCS) was a product that resulted from further processing of the P-LCN. The processes involve microwave heating of the beans at a temperature range of 90–100 °C for a period of about 15 min on a vibratory bed (this makes the cocoa bean shell puff for easy winnowing), automated roasting (at temperature range of 90–100 °C for about 20 min in a rotary evaporator), refining to over 98% fineness to obtain cocoa liquor (masse) which is further heat-treated at 80–90 °C for about 12 h in storage tanks. The liquor is later fed in batches of about 200 kg sizes into a steam-jacketed vacuum mixer, where liquor homogenisation and further heating takes place for about 10 min before final pressing to obtain cocoa butter and cocoa cake. The final heating and homogenisation are used to take the liquor from about 80 °C to about 105 °C and to ensure maturation of the liquor. This also further ensures a final rapid evaporation of residual moisture to <1% in the cocoa liquor and guarantees acceptable sterilisation of the liquor. The pressed cake is kibbled mechanically to obtain smaller sizes of the cocoa cake solid, otherwise called processed cocoa cake samples (PCCS). The factory samples were labelled process-line cocoa nibs (P-LCN) and processed cocoa cake samples (PCCS), respectively. These formed the group two samples.

2.2. Sample treatment

The samples were homogenised and ground to fine powder, using a Mouliness blender. The ground portions were kept in plastic rubbers in the freezer (−4 °C) pending analysis. The values reported for each test were averages of two or more determinations.

2.3. Determination of crude protein

Nitrogen was determined by the micro-Kjeldahl method, as described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying by 6.25.

2.4. Determination of amino acids

The amino acids profile in the cocoa samples was determined using methods described by Adeyeye and Afolabi (2004). 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 °C under vacuum in a rotary evaporator. Each residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in a plastic specimen bottle and 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 5–10 μl and about 76 min elapsed for each analysis. The column flow rate was 0.50 ml/min at 60 °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. The averages of two determinations were reported. All chemicals used were of analytical grade.

2.5. Estimation of quality of dietary protein

The essential amino acid score was 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).

Amino acid score (for both essential and non-essential amino acids) was also calculated based on whole hen's egg (Paul, Southgate, & Russel, 1976).

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), and the Leu/Ile ratios were calculated whilst the predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer, Cunningham, and Happich (1974), i.e. P-PER = −0.468 + 0.454 (Leu) − 0.105 (Tyr).

Calculations completed were the grand mean, standard deviation, coefficients of variation in percentage, correlation coefficient, regression and F-test, setting the confidence level at 95% (Christian, 1980).

3. Results and discussion

The crude protein levels of the unfermented and fermented samples are shown in Table 1. Also, the crude protein levels in the process-line cocoa nibs (P-LCN) and processed cocoa cake samples (PCCS) are shown in Table 2. The level of crude protein in fermented cocoa bean was 15.2 g/100 g which was better than the value in unfermented cocoa bean (13.6 g/100 g) by 1.58 g/100 g or 10.4%. This meant that the fermentation process had improved the level of the crude protein in the fermented sample compared to the unfermented sample. In the case of the group two samples, P-LCN had a crude protein level of 23.2 g/100 g which was better than the PCCS sample (18.4 g/100 g) by 4.8 g/100 g or 20.7%. This meant that the heat involved in the processing reduced the level of protein. This reduction 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 & Tobin, 1980). However, Ala increased in the PCCS.

The amino acids composition of the unfermented and fermented cocoa nibs is presented in Table 1. The amino acid concentrations were variously distributed amongst the two samples, and this could easily be seen both in the various samples and in the coefficient of variation percentage (CV%). Glutamic acid (Glu) was the most concentrated amino acid amongst the samples: 128 mg/g crude protein (cp) (in unfermented cocoa nibs) and 153 mg/gcp (fermented cocoa nibs). Another acidic amino acid, aspartic acid (Asp), occupied the second position in both samples with values of 100 mg/gcp (unfermented sample) and 82.5 mg/gcp (fermented sample). Cystine (Cys) was the least concentrated, in both samples, with values of 7.8 mg/gcp (unfermented cocoa nibs) and 6.9 mg/gcp (fermented nibs). The fermented cocoa nibs were richer than were unfermented cocoa nibs in the following amino acids: Lys, His, Arg, Ser, Glu, Gly, Ala, Val, Ile, Tyr and Phe, whereas Pro (or one amino acid, 5.88%) was of equivalent level (12.5 mg/gcp in both samples). This meant that the fermented cocoa nibs were 64.7% richer in the amino acids than were the unfermented nibs; whereas the unfermented sample was only better in five (or 29.4%) of the amino acids. Therefore, fermentation improved the amino acid profile of the cocoa nibs. This is particularly true for most of the essential amino acids: Lys, His, Arg, Thr, Val, Ile and Phe. The improvement of amino acid concentration by fermentation followed the trend observed in guinea corn, where steeping of the grains improved the amino acid profile over the raw and germinated samples in Arg, His, Met, Phe, Thr, Val, Ala, Cys, Gly, Pro, Ser, and Tyr (Adeyeye, 2008).

Table 1
Amino acid profiles (mg/g crude protein) of unfermented and fermented cocoa nibs from a farm location.

Amino acid	Unfermented nibs	Fermented nibs	Mean	SD	CV%
Lys*	42.0 ± 0.02	52.6 ± 0.02	47.3	7.50	15.9
His*	20.0 ± 0.00	23.3 ± 0.02	21.7	2.33	10.7
Arg*	43.6 ± 0.01	51.4 ± 0.20	47.5	5.52	11.6
Asp	100 ± 0.10	82.5 ± 0.11	91.3	12.4	13.6
Thr*	29.9 ± 0.03	23.3 ± 0.10	26.6	4.67	17.6
Ser	23.7 ± 0.01	32.6 ± 0.03	28.2	6.29	22.3
Glu	128 ± 0.20	153 ± 0.40	141	17.7	12.6
Pro	12.5 ± 0.02	12.5 ± 0.03	12.5	0.00	–
Gly	20.5 ± 0.01	32.0 ± 0.02	26.3	8.13	30.9
Ala	29.8 ± 0.20	40.1 ± 0.03	35.0	7.28	20.8
Cys	7.8 ± 0.01	6.9 ± 0.02	7.35	0.64	8.71
Val*	32.1 ± 0.10	35.1 ± 0.02	33.6	2.12	6.31
Met*	9.9 ± 0.01	8.0 ± 0.00	8.95	1.34	15.0
Ile*	21.4 ± 0.02	29.3 ± 0.20	25.4	5.59	22.0
Leu*	72.2 ± 0.30	62.4 ± 0.20	67.3	6.93	10.3
Tyr	18.6 ± 0.02	27.0 ± 0.01	22.8	5.94	26.1
Phe*	28.6 ± 0.01	36.3 ± 0.02	32.5	5.44	16.7
Try*	–[a]	–	–	–	–
Crude protein (g/100 g)	13.6 ± 0.30	15.2 ± 0.21			

* Essential amino acids.
[a] Not determined.

Table 2
Amino acid profiles (mg/g crude protein) of process-line cocoa nibs and processed cocoa cake samples from a processing industry.

Amino acid	P-LCN[a]	PCCS[b]	Mean	SD	CV%
Lys	181 ± 0.05	52.7 ± 0.03	117	90.7	77.5
His	11.7 ± 0.01	4.4 ± 0.01	8.05	5.16	64.1
Arg	46.3 ± 0.02	29.4 ± 0.20	37.9	12.0	31.7
Asp	37.4 ± 0.03	43.7 ± 0.20	40.6	4.45	11.0
Thr	15.9 ± 0.02	8.7 ± 0.01	12.3	5.09	41.4
Ser	21.4 ± 0.00	23.1 ± 0.02	22.3	1.20	5.38
Glu	37.4 ± 0.10	43.5 ± 0.02	40.5	4.31	10.6
Pro	28.1 ± 0.02	25.1 ± 0.01	26.6	2.12	7.97
Gly	2.9 ± 0.01	5.0 ± 0.01	3.95	1.48	37.5
Ala	1.5 ± 0.01	20.1 ± 0.02	10.8	13.2	122
Cys	15.3 ± 0.02	10.8 ± 0.01	13.1	3.18	24.3
Val	39.2 ± 0.03	2.4 ± 0.01	20.8	26.0	125
Met	4.3 ± 0.01	3.7 ± 0.02	4.0	0.42	10.5
Ile	63.3 ± 0.30	31.9 ± 0.05	47.6	22.2	46.6
Leu	47.2 ± 0.02	37.0 ± 0.20	42.1	7.21	17.1
Tyr	13.6 ± 0.02	12.1 ± 0.11	12.9	1.06	8.22
Phe	21.8 ± 0.02	14.8 ± 0.01	18.3	4.95	27.0
Try	–	–	–	–	–
Crude protein (g/100 g)	23.2 ± 0.20	18.4 ± 0.03			

[a] Process-line cocoa nibs.
[b] Processed cocoa cake samples.

Our results, in both the unfermented and fermented cocoa nibs, followed the trends in *Cola acuminata, Garcinia kola* and *Anacardium occidentale*, where Glu was the most concentrated amino acid and Asp was the second most concentrated in *C. acuminata* and *G. kola* (Adeyeye, Asaolu, & Aluko, 2007). Our trend in Glu and Asp agreed with the results of Olaofe, Adeyemi, and Adediran (1994) who found that Glu and Asp, respectively, were the first and second most concentrated amino acids in some oilseeds. The differences in the levels of the amino acids in the two samples were shown by the various levels of the CV%; when subjected to the F-test the differences were not significant at $p < 0.05$ since F_c (1.11) < F_t (2.35). Also, the correlation coefficient was high at $r_{0.96}$ and regression (R_c) was 3.51.

Table 2 contains the amino acid profiles for the process-line cocoa nibs (P-LCN) and processed cocoa cake samples (PCCS). In both samples, Lys was the most concentrated amino acid with values of 181 mg/gcp in P-LCN and 52.7 mg/gcp in PCCS; whilst Ile (63.3 mg/gcp) was the second most concentrated in P-LCN, it was Asp (43.7 mg/gcp) in PCCS. The following amino acids were more concentrated in P-LCN than in PCCS (mg/gcp): Lys, His, Arg, Thr, Pro, Cys, Val, Met, Ile, Leu, Tyr and Phe, or 70.6% better in amino acid

concentration. This meant that P-LCN would be a better protein food ingredient than would PCCS. Here some of CV% levels were well above 50.0 and the data, when subjected to F-test analysis, showed that significant differences existed at $p < 0.05$ between the P-LCN and PCCS amino acid profiles, since F_c (6.86) > F_t (2.35). The $r_{0.70}$ was less than the value obtained in Table 1 but the R_c was 12.4 which was much higher than the value in Table 1.

It is interesting to note the high differences in the amino acids, Lys, His, Arg, and Cys between P-LCN and PCCS samples. The availability of some amino acids, for example, Lys, Met, Arg, Try, Cys and His, is often severely impaired when the protein in the food is heated, e.g. in processing, or where it is improperly stored. This impairment occurs when the Amadori rearrangement goes beyond the deoxy-ketosyl stage due to heat treatment. This is particularly serious when intravenous drip fluids containing sugars and proteins undergo Maillard reactions during sterilisation (Muller & Tobin, 1980). Carpenter has used the susceptibility of Lys to heat damage in the presence of moisture as a basis for estimating the extent of the damage (Muller & Tobin, 1980). Although Val may be thermally stable, it may also be possible that it takes part in this type of browning reaction which will definitely reduce its concentration during heat processing.

The processed cocoa cake samples (PCCS) underwent various heat processing unit operations which lasted for 13 h or more. This would have led to a very serious heat effect on the amino acids. Looking critically at Table 2, where the values of P-LCN and PCCS are compared, wide variation existed between the essential amino acids of the two samples. For example, loss of amino acid concentration from P-LCN to PCCS goes thus (mg/g crude protein) Lys, 128 (78.7%); His, 7.3 (62.4%); Arg, 16.9 (36.6%); Thr, 7.2 (45.3%); Cys, 4.5 (29.4%); Val, 36.8 (90.9%); Met, 0.6 (1.40%); Ile, 31.4 (49.6%); Tyr, 1.5 (11.0%) and Phe, 7.0 (32.1%). These values show serious reductions in the available essential amino acids, unlike the non-essential amino acids of the PCCS. Usually, the method used for the amino acid analysis will only detect L-amino acids from animal and plant proteins that do not produce racemisation (White et al., 1973). The reasons for these serious reductions of the amino acids from P-LCN to PCCS would likely be due to Amadori rearrangement that goes beyond the deoxy-ketosyl stage and the formation of D-amino acids which are both due to high and prolonged heat treatment (Fennema, 1985; Muller & Tobin, 1980).

Various parameters are presented in Tables 3 and 4. The total amino acids (TAA) in unfermented cocoa nibs was 641 mg/gcp and it was 708 mg/gcp in fermented cocoa nibs (Table 4) whilst it was 635 mg/gcp in P-LCN and 368 mg/gcp in PCCS (Table 3). The TAA in unfermented cocoa nibs was close to the value of 659 mg/gcp in *A. occidentale* and also the value of TAA in PCCS was close to the value of 356 mg/gcp in *C. acuminaa* (Adeyeye et al., 2007). The level of TAA in fermented cocoa nibs was close to the values of 703–917 mg/gcp of dehulled samples of African yam bean (Adeyeye, 1997). The total non-essential amino acids (TNEAA) for the samples were (mg/gcp): 341 (unfermented nibs), 421 (fermented nibs) – see Table 3, 158 (P-LCN) and 183 (PCCS) – see Table 4. The TNEAA of 341–421 mg/gcp was close to the value of 327–454 mg/gcp in African yam bean (Adeyeye, 1997) and also to 323 mg/gcp in *A. occidentale* (Adeyeye et al., 2007). However, in the composition of the total essential amino acids (TEAA), there was a reversal of concentration which followed this pattern (mg/gcp, with His): 478 (P-LCN) > 300 (unfermented nibs) > 287 (fermented nibs) > 185(PCCS). On a percentage basis, this trend was not consistent amongst the samples. The percent TNEAA ranged between 53.3% (unfermented nibs) and 59.5% (fermented nibs) with a low value of CV% (7.77) (Table 3); from Table 4 it ranged from 24.8% (P-LCN) to 49.8% (PCCS) with a high value of CV% (64.8). The % TEAA (with His) ranged between 75.2% (P-LCN) and 50.2% (PCCS) with CV% of 28.2 (Table 4). These results showed that the industrial processed cake was again lower in the TEAA than in the process on line by a wide margin. The total neutral amino acids (TNAA) levels were close in (mg/gcp); 228 (unfermented nibs), 235 (fermented nibs) but low in P-LCN (74.8) and PCCS (87.2).

The TEAA in melon and gourd oilseeds with respective values of 534 mg/g and 536 mg/gcp (Olaofe et al., 1994) were reportedly higher than all of our values, that ranged between 185 mg/gcp and 478 mg/gcp; with the exception of 478 mg/gcp (P-LCN), all of our EAA values were lower than those in soybean (444 mg/gcp) (Kuri, Sundar, Kahuwi, Jones, & Rivett, 1991). Our present samples were either close to or lower than the following TEAA levels (mg/gcp): pigeon pea (452) (Nwokolo, 1987), pumpkin seed (396)

Table 3
Concentrations of essential, non-essential, acidic, neutral, sulphur, aromatic (mg/g crude protein) of unfermented and fermented cocoa nibs.

Amino acid	Unfermented Nibs	Fermented nibs	Mean	SD	CV%
Total amino acid (TAA)	641	708	675	43.4	6.43
Total non-essential amino acid (TNEAA)	341	421	381	56.6	14.9
Total essential amino acid (TEAA)					
–With His	300	287	294	9.19	3.13
–No His	280	263	272	12.0	4.41
% TNEAA	53.3	59.5	56.4	4.38	7.77
% TEAA					
–With His	46.8	40.5	43.7	4.5	10.2
–No His	43.6	37.2	40.4	4.53	11.2
Total neutral amino acid (TNAA)	307	346	327	27.6	8.44
% TNAA	47.9	48.8	48.4	0.64	1.32
Total acidic amino acid (TAAA)	228	235	232	4.95	2.13
% TAAA	35.6	33.2	34.4	1.7	4.94
Total basic amino acid (TBAA)	106	127	117	14.8	12.6
% TBAA	16.5	18.0	17.3	1.06	6.13
Total sulphur amino acid (TSAA)	17.7	14.9	16.3	1.98	12.1
% TSAA	2.76	2.11	2.44	0.46	18.9
% Cys in TSAA	44.1	46.3	45.2	1.56	3.45
Total aromatic amino acid (TArAA)	47.2	63.3	55.3	11.4	20.6
% TArAA	7.36	8.94	8.15	1.12	13.7
P-PER*	3.55	2.55	3.05	0.71	23.3
Leu/Ile ratio	3.37	2.13	2.75	0.88	32.0
Leu-Ile (difference)	50.8	33.1	42.0	12.5	29.8
% Leu-Ile	70.4	53.0	61.7	12.3	19.9

* Predicted protein efficiency ratio.

Table 4
Concentrations of essential, non-essential, acidic, neutral, sulphur, aromatic amino acids (mg/g crude protein) of P-LCN and PCCS.

Amino acid	P-LCN*	PCCS*	Mean	SD	CV%
Total amino acid (TAA)	635	368	502	189	37.6
Total non-essential amino acid (TNEAA)	158	183	171	17.7	10.4
Total essential amino acid (TEAA)					
–With His	478	185	332	207	62.3
–No His	466	181	324	202	62.3
% TNEAA	24.8	49.8	27.3	17.7	64.8
% TEAA					
–with His	75.2	50.2	62.7	17.7	28.2
–no His	73.3	49.0	61.2	17.2	28.1
Total neutral amino acid (TNAA)	322	195	259	89.8	34.7
% TNAA	50.7	52.9	51.8	1.56	3.01
Total acidic amino acid (TAAA)	74.8	87.2	81.0	8.77	10.8
% TAAA	11.8	23.7	17.8	8.4	47.2
Total basic amino acid (TBAA)	239	86.5	163	108	66.3
% TBAA	37.6	23.5	30.6	9.97	32.6
Total sulphur amino acid (TSAA)	19.6	14.5	17.1	3.61	21.1
% TSAA	3.09	3.94	3.52	0.60	17.0
% Cys in TSAA	78.1	74.5	76.3	2.55	3.34
Total aromatic amino acid (TArAA)	35.4	26.9	31.2	6.01	19.3
% TArAA	5.57	7.31	6.44	1.23	19.1
P-PER*	2.47	2.02	2.25	0.32	14.2
Leu/Ile ratio	0.75	1.16	0.96	0.29	30.2
Leu-Ile (difference)	−16.1	5.1	10.6	7.78	73.4
% Leu-Ile	−34.1	13.8	24.0	14.4	60.0

* See Table 2.

(Aisegbu, 1987), cowpea (426) (Olaofe, Umar, & Adediran, 1993) and *Cajanus cajan* (436) (Oshodi, Olaofe, & Hall, 1992). This meant that unfermented nibs, fermented nibs and PCCS protein in the samples were of lower quality than those in cowpea, soybean and pigeon pea. However, whilst Cys was 0.0 mg/gcp in melon, pumpkin seed and gourd seed (Olaofe et al., 1994) and 11.3 mg/gcp in *A. occidentale*, 2.5 mg/gcp in *G. kola* and 4.5 mg/gcp in *C. acuminata* (Adeyeye et al., 2007), it was (mg/gcp): in unfermented nibs (17.7), fermented nibs (14.9), P-LCN (19.6) and PCCS (14.5). Generally, most of our results were better in many of the amino acids (essential and non-essential) than was pumpkin seed (Olaofe et al., 1994).

Whilst it is known that cystine can spare part of the requirement for methionine, FAO/WHO/UNICEF (1985) does not give any indication of the proportion of total sulphur amino acids that can be met by Cys. For the 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: 44.1% (unfermented nibs), 46.3% (fermented nibs) – see Table 3; 78.1 (P-LCN) and 74.5 (PCCS) – see Table 4. Whilst the Cys/TSAA% in unfermented and fermented nibs followed the trend in *G. kola* (37.8%) and *C. acuminata* (44.3%) (Adeyeye et al., 2007), as well as in animals: 36.3% (*Macrotermes bellicosus*) (Adeyeye, 2005a), 25.6% (*Zonocerus variegatus*) (Adeyeye, 2005b), 35.5% (*Archachatina marginata*), 38.8% (*Archatina archatina*) and 21.0% (*Limicolaria* sp.) (Adeyeye & Afolabi, 2004), the Cys/TSAA% in P-LCN and PCCS followed the trend in *A. occidentale* (50.5%) (Adeyeye et al., 2007), coconut endosperm (62.9%) (Adeyeye, 2004), raw guinea corn (58.9%), steeped guinea corn (72.0%) and germinated guinea corn (71.1%) (Adeyeye, 2008). This meant that, whilst both unfermented and fermented cocoa nibs behaved like animal proteins under these conditions, the P-LCN and PCCS behaved like plant proteins. 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 better in the unfermented (3.55) and fermented (2.55) cocoa nibs than in the P-LCN (2.47) and PCCS (2.02) samples. The experimentally determined PER usually ranged from 0.0 for a very poor protein to a maximum possible of just over 4 (Muller & Tobin, 1980). These results showed that P-LCN and PCCS would likely be less utilised in the body than would the other two samples.

The Leu/Ile ratio values ranged as follows: 3.37 (unfermented nibs), 2.13 (fermented nibs), 0.75 (P-LCN) and 1.16 (PCCS). From Table 1, the level of Leu was more than twice that of Ile in unfermented and fermented nibs whilst, in Table 2, the level of Leu was just above one half that of Ile in P-LCN and slightly above Ile in PCCS. 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).

High Leu in the diet impairs tryptophan and niacin metabolism and is responsible for niacin deficiency in sorghum eaters (Belavady, Srikantia, & Gopalan, 1963) . This leads to 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) suggested that 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. Experiments in dogs have shown that animals fed sorghum proteins (with less than 110 mg/gcp of Leu) did not suffer from nicotinic acid deficiency (Belavady & Udayasekhara Rao, 1979). None of our samples had levels of Leu up to 110 mg/gcp.

Table 5 contains the amino acid scores, based on the provisional amino acids, for the unfermented and fermented cocoa nibs whilst Table 6 contains the scores of P-LCN and PCCS, based on the same formula. In Table 5, the limiting amino acids for both unfermented and fermented cocoa nibs were Met + Cys with respective values of 0.51 (unfermented nibs) and 0.43 for fermented nibs. The entire CV% was low and no significant differences existed between the two samples at $p < 0.05$ (F-test). Therefore, in order to fulfil the day's needs for the EAA in unfermented cocoa nibs, 100/51 or 1.96 times as much unfermented nibs would have to be eaten when it is the sole protein in the diet; in fermented nibs, it would be 100/43 or 2.33 times the protein level. In Table 6, Thr was the limiting

Table 5
Essential amino acid scores of the unfermented and fermented cocoa nibs based on provisional amino acid scores.

Amino acid	Unfermented nibs	Fermented nibs	Mean	SD	CV%
Ile	0.54	0.73	0.64	0.13	20.3
Leu	1.07	0.89	0.98	0.13	13.3
Lys	0.76	0.96	0.86	0.14	16.3
Met + Cys	0.51	0.43	0.47	0.06	12.8
Phe + Tyr	0.79	1.06	0.93	0.19	20.4
Thr	0.76	0.58	0.67	0.13	19.4
Try	–	–	–	–	–
Val	0.64	0.7	0.67	0.04	5.97

Table 6
Essential amino acid scores of the P-LCN and PCCS based on provisional amino acid scores.

Amino acid	P-LCN*	PCCS*	Mean	SD	CV%
Ile	1.58	0.80	1.19	0.55	46.2
Leu	0.67	0.53	0.60	0.10	16.7
Lys	3.28	0.96	2.12	1.64	77.4
Met + Cys	0.56	0.41	0.49	0.11	22.4
Phe + Tyr	0.59	0.45	0.52	0.11	19.2
Thr	0.40	0.22	0.31	0.13	41.9
Try	–	–	–	–	–
Val	0.78	0.05	0.42	0.52	124

Table 7
Amino acid scores of the four samples based whole hen's egg.

Amino acid	Unfermented nibs	Fermented nibs	P-LCN	PCCS
Lys	0.68	0.85	2.92	0.85
His	0.83	0.97	0.49	0.18
Arg	0.71	0.84	0.76	0.48
Asp	0.93	0.77	0.35	0.41
Thr	0.59	0.46	0.31	0.17
Ser	0.3	0.41	0.27	0.29
Glu	1.07	1.28	0.31	0.36
Pro	0.33	0.33	0.74	0.66
Gly	0.68	1.07	0.1	0.17
Ala	0.55	0.74	0.028	0.37
Cys	0.43	0.38	0.85	0.6
Val	0.43	0.47	0.52	0.032
Met	0.31	0.25	0.13	0.12
Ile	0.38	0.52	1.13	0.57
Leu	0.87	0.75	0.57	0.45
Tyr	0.47	0.68	0.34	0.3
Phe	0.56	0.71	0.43	0.29
Try	–	–	–	–

amino acid in P-LCN (0.40) and Val in PCCS (0.05); their correction values would be 2.50 times the protein in P-LCN and 20 times in PCCS. There were CV% levels of 124 (Val), 77.4 (Lys) and 46.2 (Ile) and significant differences existed between the P-LCN and PCCS samples at $p < 0.05$ when subjected to F-test analysis.

Table 7 contains the amino acid scores of the four samples based on whole hen's egg amino acid profile. Serine was the limiting amino acid in unfermented nibs (0.30), Met in fermented nibs (0.25), Ala in P-LCN (0.028) and Val in PCCS (0.032). The respective corrections would then be 100/30 or 3.3 (unfermented nibs), 100/25 or 4.0 (fermented nibs), 100/2.8 or 35.7 (P-LCN) and 100/3.2 or 31.3 (PCCS) times the protein of each sample where they serve as the sole protein sources.

The data obtained for TNEAA, TEAA and EAA scores were all subjected to the F-test as follows: TNEAA/TNEAA, TEAA/TNEAA and EAA score/EAA score for the pairs of unfermented/fermented nibs and P-LCN/PCCS setting the $p < 0.05$ (Christian, 1980). The following results were obtained. In unfermented/fermented cocoa nibs, TNEAA/TNEAA $F_c < F_t$ and in P-LCN/PCCS result was $F_c < F_t$, results not significantly different; in TEAA/TNEAA in unfermented nibs, $F_c > F_t$; in fermented nibs, $F_c > F_t$, in P-LCN, $F_c > F_t$ meaning all the values were significantly different but, in PCCS, $F_c < F_t$; hence results were not significantly different. For the EAA score/EAA score, in unfermented/fermented nibs, $F_c < F_t$, but not significant; for P-LCN/PCCS, $F_c > F_t$, and results were significantly different at $p < 0.05$.

In conclusion, the findings of this study showed that there was a more positive build up of AA in fermented nibs than in unfermented nibs. In the processing, about 40% of the AA was destroyed; this might have resulted from the series of reactions involving amino acids, nitrates and antioxidants during the heat processing of cocoa. Finally, more cake (PCCS) would be required for complementation/fortification than would have been used if the unprocessed nibs were to be used. Also the differences observed in the fermented and unfermented samples (group one) and P-LCN and PCCS (group two) could be due to planting material, climate, varieties, application of fertiliser, heat treatments and storage between the group one and two samples.

Acknowledgement

The authors are grateful to Dr. Segun Awolumate, the Managing Director of Co-operative Cocoa Products Limited, Akure, Nigeria for his collaborative support.

References

Adeyeye, E. I. (1997). Amino acid composition of six varieties of dehulled African yam bean (*Sphenostylis stenocarpa*) flour. *International Journal of Food Sciences and Nutrition, 48*, 345–351.
Adeyeye, E. I. (2004). The chemical composition of liquid and solid endosperm of ripe coconut. *Oriental Journal of Chemistry, 20*(3), 471–476.
Adeyeye, E. I. (2005a). The composition of the winged termites, *Macrotermes bellicosus*. *Journal of Chemical Society of Nigeria, 30*(2), 145–149.
Adeyeye, E. I. (2005b). Amino acid composition of variegated grasshopper, *Zonocerus variegatus*. *Tropical Science, 45*(4), 141–143.
Adeyeye, E. I. (2008). The intercorrelation of the amino acid quality between raw, steeped and germinated guinea corn (*Sorghum bicolor*) grains. *Bulletin of the Chemical Society of Ethiopia, 22*(1), 11–17.
Adeyeye, E. I., & Afolabi, E. O. (2004). Amino acid composition of three types of land snails consumed in Nigeria. *Food Chemistry, 85*, 535–539.
Adeyeye, E. I., Asaolu, S. S., & Aluko, A. O. (2007). Amino acid composition of two masticatory nuts (*Cola acuminata* and *Garcinia kola*) and a snack nut (*Anacardium occidentale*). *International Journal of Food Sciences and Nutrition, 58*(4), 241–249.
Aisegbu, J. E. (1987). Some biochemical evaluation of fluted pumpkin seed. *Journal of the Science of Food and Agriculture, 40*, 151–155.
Akinyeye, R. O. (1999). *Causes of capacity under-utilization in cocoa processing industries in Nigeria*. Akure, Nigeria: MBA Thesis, Federal University of Technology.
Alsmeyer, R. H., Cunningham, A. E., & Happich, M. L. (1974). Equations to predict PER from amino acid analysis. *Food Technology, 28*, 34–38.
Belavady, B., & Udayasekhara Rao, P. (1979). Leucine and isoleucine content of jowar and its pellagragenicity. *Indian Journal of Experimental Biology, 17*, 659–661.
Belavady, B., Srikantia, S. G., & Gopalan, C. (1963). The effect of oral administration of leucine on the metabolism of tryptophan. *Biochemical Journal, 87*, 652–655.
Christian, G. D. (1980). *Analytical chemistry* (3rd ed.). New York, USA: John Wiley and Sons Inc.
FAO (1995). *Sorghum and millets in human nutrition.*. Rome, Italy: FAO Food Nutrition Series, No. 27, Food and Agriculture Organisation of the United Nations.
FAO/WHO (1973). *Energy and protein requirements*. Geneva, Switzerland: Technical Report Series No. 522, WHO.
FAO/WHO (1991). *Protein quality evaluation*. Rome, Italy: Report of Joint FAO/WHO Expert Consultation, FAO Food and Nutrition Paper 51.
FAO/WHO/UNICEF (1985). *Energy and protein requirement*. Geneva, Switzerland: WHO Technical Report Series No. 724, WHO.
Fennema, O. R. (1985). *Principles of food science* (2nd ed.). New York: Marcel Dekker, Inc..
International Cocoa and Commodities Organisation (2000). *ICCO celebration of cocoa 2000*. London: K.P. Partners in Publishing.
Kuri, Y. E., Sundar, R. K., Kahuwi, C., Jones, G. P., & Rivett, D. E. (1991). Chemical composition of *Monerdica charantis* L. fruits. *Journal of Agricultural and Food Chemistry, 39*, 1702–1703.
Minife, B. W. (1989). *Chocolate, cocoa and confectionary* (3rd ed.). London: Chapman and Hall.

Morgan, J. (1994). Chocolate: A flavour and texture unlike any other. *American Journal of Clinical Nutrition, 60*(6), 1065–10675.
Muller, H. E., & Tobin, G. (1980). *Nutrition and food processing*. London: Crown Helm.
Nwokolo, E. (1987). Nutritional Evaluation of Pigeon Pea meal. *Plant Foods for Human Nutrition, 37*, 283–290.
Olaofe, O., Adeyemi, F. O., & Adediran, G. O. (1994). Amino acid and mineral compositions and functional properties of some oil seeds. *Journal of Agricultural and Food Chemistry, 42*, 878–881.
Olaofe, O., Oladeji, E. O., & Ayodeji, O. E. (1987). Metal contents of some cocoa beans produced in Ondo State, Nigeria. *Journal of the Science of Food and Agriculture, 41*, 241–244.
Olaofe, O., & Onajeta, C. C. (1986). Quality assessment of some cocoa-based beverages in the Nigerian market. *Nigerian Journal of Nutrition Science, 7*(2), 81–85.
Olaofe, O., Umar, Y. O., & Adediran, G. O. (1993). The effect of nematicides on the nutritive value and functional properties of cowpea seeds (*Vigna unguicalata* L. walp). *Food Chemistry, 46*(4), 337–342.
Opeke, L. K. (1992). *Tropical tree crops*. Ibadan: Spectrum Books Limited.
Oshodi, A. A., Olaofe, O., & Hall, G. M. (1992). Amino acid, fatty acid and mineral composition of pigeon pea (cajanus cajan). *International Journal of Food Sciences and Nutrition, 42*, 187–191.
Paul, A. A., Southgate, D. A. T., & Russel, J. (1976). *First supplement to Mclance and Widdowson's the composition of Foods*. London, UK: HMSO.
Pearson, D. (1976). *Chemical analysis of foods* (7th ed.). London: J.A. Churchhill.
Rambali, B., Van Andel, I., Schenk E., Wolterink, G., Van de Werken, G., Stevenson, H., & Vleenming, W. (2002). The contribution of cocoa additive to cigarette smoking addiction. *RIVM report* 650270002/2002.
The Biscuit, Cake, Chocolate, Confectionary Alliance (BCCCCA) (1996). *Cocoa beans, chocolate manufacturers' quality requirements* (4th ed.). London: BCCCCA.
White, A., Handler, P., & Smith, E. L. (1973). *Principles of biochemistry* (5th ed.). New York: McGraw-Hill, Inc.