

Amino acid profile of raw and locally processed seeds of *Prosopis africana* and *Ricinus communis*: potential antidotes to protein malnutrition

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ABSTRACT

Background: Increasing incidence of malnutrition occasioned by high incidence of hunger, worsening food situation in the world, insufficient availability and high cost of animal protein sources, has necessitated extensive research into and use of alternative plant protein sources especially underexploited leguminous seeds.

Methods: Flours from raw, boiled and fermented seeds of *Prosopis africana* and *Ricinus communis* were evaluated for crude protein and amino acid (AA) profiles, and their protein qualities determined.

Results: Fermentation improved the protein contents of raw seeds of *P. africana* and *R. communis* by 18.70% and 3.95% respectively. In the raw and fermented *P. africana* seeds, glutamate at 132.60 ± 1.30 and 182.70 ± 3.02 mg/g crude protein (mg/gcp) was the most abundant amino acid (AA), while leucine (62.80 ± 0.60 and 79.50 ± 2.01 mg/gcp) was the most concentrated essential amino acid (EAA). Aspartate (151.90 ± 2.01 and 170.10 ± 2.00 mg/gcp) and arginine (72.80 ± 2.01 and 78.60 ± 2.00 mg/gcp) were the most concentrated and abundant non-essential amino acid (NEAA) and EAA in the raw and fermented samples of *R. communis* respectively. The total AA concentrations (mg/gcp) of raw and fermented *P. africana* were 733.00 and 962.60 respectively, while those of *R. communis* were 823.50 and 894.10 respectively. The total EAA contents (mg/gcp) for *P. africana* were 311.00 (raw) and 404.50 (fermented), and for *R. communis*; 401.10 (raw) and 430.30 (fermented). Threonine was the limiting EAA in raw and fermented *P. africana*, whereas lysine was the limiting EAA in *R. communis* raw sample. Fermentation significantly ($p < 0.05$) increased the individual AA compositions of *P. africana* and *R. communis* by 94% and 53% respectively, while boiling reduced these parameters significantly ($p < 0.05$) by 47% and 82% respectively.

Conclusion: *P. africana* and *R. communis* seeds are potentially important plant sources of protein and essential amino acids, and so could be of great importance in combating malnutrition and food security problems generally.

Key words: Amino acid score; condiments; crude protein; fermentation; legumes; nutrition.

INTRODUCTION:

Malnutrition is directly responsible for over 300,000 deaths per year in developing countries and contributes indirectly to over half the deaths in children worldwide [1]. It is the consequence of a range of factors that are often related to poor food quality, insufficient food intake and severe infectious diseases, or most times, combinations of the three [2]. Protein-energy malnutrition is a direct consequence of poor food quality. It refers to an imbalance between the supply of protein or its metabolic building blocks, amino acids, and the body's demand for them to ensure optimal growth and function. Unfortunately, protein-rich foods are in short supply, and thus not within the reach of low-income households who form the bulk of the population in developing countries [3]. To combat these protein supply problems, current researches have focused on vast numbers of less familiar plant resources existing in the wild [4]. Considering the high incidence of hunger occasioned by the worsening food situation in the world, the insufficient availability and high cost of animal protein sources where available, there is then the need for extensive research into alternative plant protein sources especially underexploited leguminous seeds that are commonly available but largely ignored.

Leguminous seeds are known good sources of plant proteins and are traditionally available but most of their values are rarely appreciated. Among such available but neglected legumes are *Prosopis africana* and *Ricinus communis*.

Prosopis africana is a leguminous plant of the Fabaceae family. It is a flowering plant that is locally called "kiriya" in Hausa, "okpehe" in Idoma and "gbaaye" in Tiv languages of Nigeria. It was originally found in the Sahel and Savannah forests of Senegal to Ethiopia. The trees of *P. africana* are common in the middle belt and northern parts of Nigeria [5]. It grows to a height of 4-20 m, with slightly rounded buttress roots and dark stem bark, which is scaly, slash and has white streaks. It flowers once a year. The fruits are smooth, hard pod-like capsules having banana fruit shape and is brown-black in colour. The seeds are many, tiny and very hard, brownish-black in colour with high resistance to water and insect attack. It is traditionally used for formulation of animal feeds and preparation of local condiments through boiling and fermentation processes [5].

Ricinus communis, commonly called Castor bean, is a native plant in Ethiopian region of tropical East Africa, and warm temperate regions of the world. It belongs to the Euphorbiaceae family and has many vernacular names; "ogiri" (i.e. melon) in Igbo and "giga" in Tiv languages of Nigeria, "carapaterior" in Brazil and "galo" in Ethiopia [6]. Castor plant is a robust annual that may grow up to 2.5m in one season with full sunlight, heat and adequate moisture. The flowers occur most of the year in dense terminal clusters. The spiny seed pods are made up of three carpel which split apart at maturity, releasing shiny seeds with intricate designs [6]. The seeds

are extremely poisonous but have numerous economic and medical importance [6,7]. They are used for preparation of traditional condiments [8].

Condiments are substances used to give flavour to food or that is eaten with food. Traditionally, in the area of study, they are made from fermented seeds and are used mostly by the poor as low-cost protein sources in food. It has been reported that fermentation as a process improves flavour and taste, and has been described as a processing method used in homes to improve nutritional quality of foods [9,10]. This study will provide data on the protein quality as well as the effects of traditional processing techniques, viz: boiling and fermentation, on these two leguminous seeds, which are hitherto neglected and to the best of our knowledge, have not been properly documented in the study area.

MATERIALS AND METHODS:

Collection and Preparation of Seed Samples: Apparently healthy seed samples of *Prosopis africana* and *Ricinus communis* were purchased from Makurdi Main Market, Benue State, Nigeria. They were identified by a Plant Taxonomist at the Department of Forestry and Wildlife Technology, Federal University of Technology, Owerri, Nigeria, where voucher specimens were deposited.

Each of the seed samples was divided into three portions and processed as follows:

Raw Seed Samples: One portion (500g) of each seed sample was dried to a constant weight in an oven (Gallenkamp model III-100) at 60⁰C, de-shelled, ground into fine powder using a manual grinder, sieved through a No. 20 mesh sieve and stored in a dry, airtight, labeled glass container in a refrigerator at 4⁰C prior to analysis.

Boiled Seed Samples: The second portion (500g) of each seed sample was boiled in water at 100⁰C for 12 h using a gas cooker, de-shelled and ground with a manual grinder into a paste. The paste was dried in the oven at 60⁰C to a constant weight and stored in another set of labeled glass container in a refrigerator at 4⁰C until analysis.

Fermented Seed Samples: The third portion (500g) of each seed sample was boiled for 12 h using a gas cooker, de-shelled and ground with a manual grinder into a paste. Each paste was wrapped in a muslin cloth, covered in a pot and allowed to ferment at room temperature (\approx 30⁰C) for 72 h. At the end of the fermentation period, each sample was dried in the oven at 60⁰C to a constant weight and stored in an appropriately labeled glass container in a refrigerator at 4⁰C until analysis.

Determination of Protein and Amino Acid Compositions: The nitrogen content of the three ground seed sample portions was determined by the standard micro-Kjeldahl method, as described by Pearson [11], and the percentage nitrogen obtained was converted to crude protein by multiplying by 6.25 [11].

Initially, 200 mg of each ground seed sample was defatted with chloroform/methanol mixture in a ratio of 1:1. Then, 30 mg of the defatted sample was put into a glass ampoule, 7 ml of 6M HCl was added and oxygen expelled by passing nitrogen into the ampoule. The sealed ampoule was put in the oven at 105⁰C for 22 h, allowed to cool and filtered. The filtrate was then evaporated to dryness at 40⁰C under vacuum in a rotary evaporator. The residue was dissolved with 5ml acetate buffer (pH 2.0) and loaded into the amino acid analyzer where the amino acid

compositions of the seed samples were determined by Ion Exchange Chromatographic (IEC) method using the Technicon Sequential Multisample Amino Acid Analyzer (Technicon Instruments Corporation, New York).

Estimation of Seed Samples Protein Quality: To estimate the quality of dietary protein in the seed samples, the Total Essential Amino Acid (TEAA), Total Non-Essential Amino Acid (TNEAA), Total Sulphur Amino Acid (TSAA), percentage of cystine in TSAA (%Cys/TSAA), Total Aromatic Amino Acid (TAraA), Leu/Ile ratio, etc, were calculated as earlier described [12]. The Predicted Protein Efficiency Ratio (P-PER) was also determined using the equation of Alsmeyer *et al.*, [13] i.e. $P\text{-PER} = -0.468 + 0.454 (\text{Leu}) - 0.105 (\text{Tyr})$. The essential amino acid scores of the seed samples were also calculated using the formula: Amino acid score = amount of amino acid per test protein (mg/g) / amount of amino acid per protein in reference pattern (mg/g) [12].

Statistical Analysis: Data generated were organized into means and standard deviations. Their coefficient of variations in percentages were calculated. Inferences were taken at confidence level of 95% using one-way analysis of variance and Tukey posthoc tests [14], with the aid of a computer-based statistical package, GraphPad Prism 5.3 (GraphPad Software Inc. USA).

RESULTS AND DISCUSSION:

The crude protein contents of the raw, boiled, and boiled + fermented (simply represented as fermented) seed samples of *Prosopis africana* and *Ricinus communis* are shown in Figure 1.

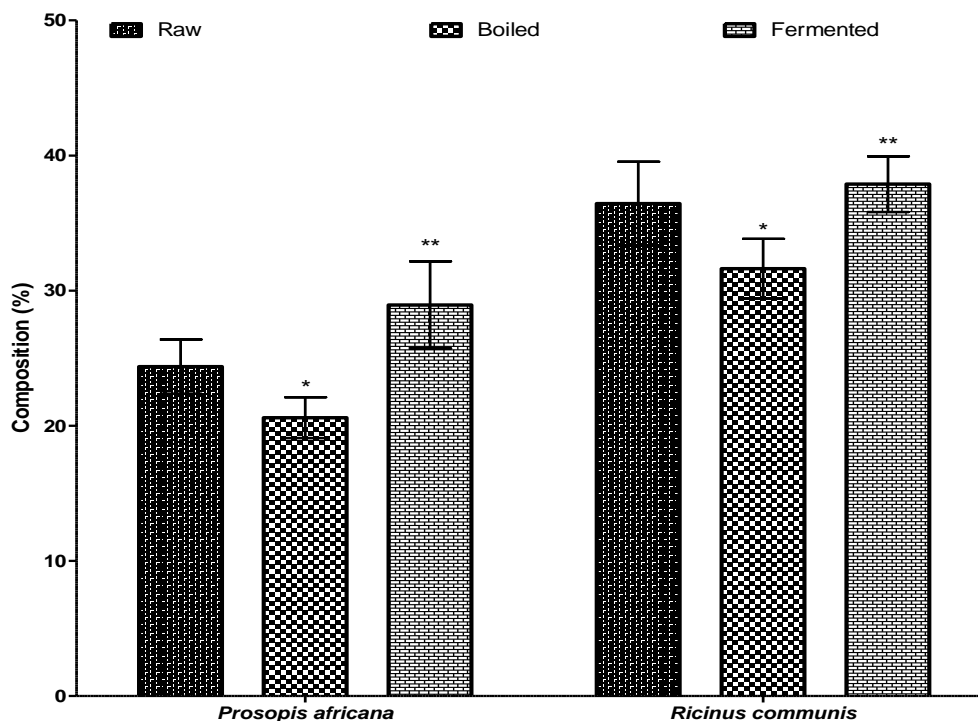


Figure 1: Crude protein composition (%) of raw, boiled and fermented seed samples of *Prosopis africana* and *Ricinus communis*. * (or **) = Value significantly ($p < 0.05$) reduced (or increased) in comparison with respective raw sample value.

It shows that the crude protein content of the raw seeds were 24.38 ± 2.01 g/100g and 36.44 ± 3.10 g/100g respectively. Boiling, as a processing technique significantly ($p < 0.05$) reduced the seeds' crude protein content by 15.50% and 13.20% respectively, while the combined processes of boiling and fermentation significantly ($p < 0.05$) increased it by 18.70% and 3.95% respectively. These observations are in consonance with earlier reported raw seed crude protein levels of 25.74% and 23.6% in *P. africana* [15,5], and 21–48% in *R. communis* [16]. Generally, the crude protein levels obtained in this present study are comparable to those of *Sesamum indicum* ($24.74 \pm 0.56\%$), fluted pumpkin (28.7%) and African oil bean (36.2%) seeds [17,18], but higher than those of several reported common seeds and fruits [4,19]. Boiling and fermentation have been previously reported to have contrasting effects on protein contents of food materials. While boiling reduces crude protein content, fermentation was reported to improve nutritional quality [4,20]. The positive effect of fermentation is associated with the action of microflora and enzymes which breakdown complexes enhancing release and availability of proteins. On the other hand, the reduction in protein content associated with boiling for up to 12 h in this study may be due to the heat involved which leads to Maillard reactions and subsequent Amadori rearrangements making the protein and its amino acids significantly unavailable [21].

The amino acid compositions of the raw, boiled and fermented seed samples of *P. africana* and *R. communis* are presented in Tables 1 and 2. The amino acid concentrations of the two seed crops were variously distributed amongst the three samples (i.e. raw, boiled and fermented) of each crop, and these are apparent from results of the various samples and in their coefficient of variation percentages (CV%). Glutamic acid (Glu) at 132.60, 131.50 and 182.70 mg/g crude protein (mg/gcp) was the most concentrated amino acid amongst the three samples of *P. africana*, while aspartic acid (Asp) at 151.90, 141.90 and 170.10 mg/gcp was the most concentrated amino acid in the respective samples of *R. communis*. In both seed crops, the remaining acidic amino acid in each case, occupied the second position in terms of abundance.

Table 1: Amino acid profiles (g/100g crude protein) of raw, boiled and fermented seed samples of *Prosopis africana*

| Amino acids | Raw | Boiled | Fermented | Mean | SD | CV% |
|----------------------------------|---------------------|---------------------|---------------------|--------|-------|-------|
| Essential amino acids | | | | | | |
| Lys | 40.60 ± 1.10^a | 34.60 ± 0.80^b | 47.20 ± 0.30^c | 40.80 | 6.30 | 15.45 |
| His | 16.30 ± 0.30^a | 11.90 ± 0.10^b | 25.00 ± 0.10^c | 17.73 | 6.67 | 37.59 |
| Arg | 41.60 ± 1.00^a | 30.00 ± 0.50^b | 49.00 ± 0.51^c | 40.20 | 9.58 | 23.82 |
| Thr | 24.60 ± 0.61^a | 23.60 ± 0.20^a | 27.71 ± 0.10^b | 24.63 | 3.05 | 12.38 |
| Val | 40.30 ± 0.80^a | 38.20 ± 0.31^a | 53.20 ± 0.60^b | 43.90 | 8.12 | 18.50 |
| Met | 10.40 ± 0.10^a | 4.20 ± 0.10^b | 13.10 ± 0.20^c | 9.23 | 4.56 | 49.42 |
| Ile | 32.80 ± 1.22 | 25.20 ± 0.21^b | 42.60 ± 0.61^c | 33.53 | 8.72 | 26.01 |
| Leu | 62.80 ± 0.60^a | 60.40 ± 0.81^a | 79.50 ± 2.00^b | 67.57 | 10.40 | 15.40 |
| Phe | 41.60 ± 3.01^a | 40.60 ± 0.21^a | 67.20 ± 1.21^b | 49.80 | 15.08 | 30.28 |
| Non-essential amino acids | | | | | | |
| Glu | 132.60 ± 1.30^a | 131.50 ± 1.01^a | 182.70 ± 3.02^b | 148.90 | 29.25 | 19.64 |

| | | | | | | |
|-----|---------------------------|---------------------------|---------------------------|-------|-------|-------|
| Ser | 46.80 ± 0.60 ^a | 45.90 ± 0.59 ^a | 64.40 ± 1.01 ^b | 52.37 | 10.43 | 19.92 |
| Asp | 66.80 ± 1.20 ^a | 51.20 ± 0.41 ^a | 81.30 ± 1.03 ^a | 66.43 | 15.05 | 22.66 |
| Pro | 38.00 ± 0.31 ^a | 36.10 ± 0.20 ^b | 41.90 ± 0.50 ^c | 38.67 | 2.96 | 7.65 |
| Gly | 33.50 ± 0.91 ^a | 30.10 ± 0.12 ^b | 46.00 ± 0.42 ^c | 36.53 | 8.37 | 22.92 |
| Ala | 49.70 ± 0.60 ^a | 23.20 ± 0.21 ^b | 59.30 ± 0.91 ^c | 44.07 | 18.70 | 42.43 |
| Cys | 21.20 ± 0.21 ^a | 20.50 ± 0.11 ^a | 41.90 ± 0.20 ^b | 27.87 | 12.16 | 43.63 |
| Tyr | 33.40 ± 0.30 ^a | 32.20 ± 0.23 ^a | 40.60 ± 0.51 ^b | 35.40 | 4.54 | 12.83 |

Values (mean ± standard of duplicate determinations) with different superscripts per row are significantly ($p < 0.05$) different. SD, Standard deviation; CV%, coefficient of variation percentage.

Table 2: Amino acid profiles (g/100g crude protein) of raw, boiled and fermented seed samples of *Ricinus communis*

| Amino acids | Raw | Boiled | Fermented | Mean | SD | CV% |
|----------------------------------|----------------------------|----------------------------|----------------------------|--------|-------|-------|
| Essential amino acids | | | | | | |
| Lys | 44.00 ± 0.20 ^a | 41.30 ± 0.50 ^b | 52.90 ± 0.71 ^c | 46.07 | 6.07 | 13.18 |
| His | 16.90 ± 0.10 ^a | 13.10 ± 0.10 ^b | 21.30 ± 0.32 ^c | 17.10 | 4.10 | 24.00 |
| Arg | 72.80 ± 2.01 ^a | 53.20 ± 1.10 ^b | 78.60 ± 2.00 ^a | 68.20 | 13.31 | 19.52 |
| Thr | 37.20 ± 0.21 ^a | 36.50 ± 0.31 ^a | 38.20 ± 0.12 ^a | 37.30 | 0.85 | 2.29 |
| Val | 48.70 ± 0.30 ^a | 40.50 ± 0.12 ^b | 50.10 ± 1.01 ^a | 46.43 | 5.19 | 11.17 |
| Met | 27.60 ± 0.21 ^a | 23.40 ± 0.11 ^b | 29.00 ± 0.21 ^a | 26.67 | 2.91 | 10.93 |
| Ile | 37.00 ± 0.50 ^a | 26.40 ± 0.32 ^b | 38.00 ± 1.03 ^a | 33.80 | 6.43 | 19.02 |
| Leu | 64.50 ± 2.01 ^a | 60.00 ± 1.40 ^a | 66.50 ± 2.33 ^a | 63.67 | 3.33 | 5.23 |
| Phe | 52.40 ± 1.07 ^a | 43.40 ± 0.81 ^b | 55.70 ± 1.21 ^a | 50.50 | 6.37 | 12.61 |
| Non-essential amino acids | | | | | | |
| Glu | 102.20 ± 4.10 ^a | 92.80 ± 2.50 ^a | 103.50 ± 2.01 ^a | 99.50 | 5.84 | 5.87 |
| Ser | 31.90 ± 0.21 ^a | 21.50 ± 0.32 ^b | 32.90 ± 0.21 ^c | 28.77 | 6.31 | 21.95 |
| Asp | 151.90 ± 2.01 ^a | 141.90 ± 3.03 ^b | 170.10 ± 2.00 ^c | 154.60 | 14.30 | 9.25 |
| Pro | 29.80 ± 0.13 ^a | 17.00 ± 0.21 ^b | 32.90 ± 0.80 ^c | 26.57 | 8.43 | 31.73 |
| Gly | 10.80 ± 0.10 ^a | 4.00 ± 0.11 ^b | 16.80 ± 0.20 ^c | 10.53 | 6.40 | 60.80 |
| Ala | 52.50 ± 0.30 ^a | 37.10 ± 0.39 ^b | 56.30 ± 0.61 ^c | 48.63 | 10.17 | 20.91 |
| Cys | 17.50 ± 0.21 ^a | 10.50 ± 0.09 ^b | 24.00 ± 0.19 ^c | 17.33 | 6.75 | 38.95 |
| Tyr | 25.80 ± 0.23 ^a | 25.80 ± 0.11 ^b | 27.30 ± 0.20 ^c | 26.30 | 0.87 | 3.29 |

Values (mean ± standard of duplicate determinations) with different superscripts per row are significantly ($p < 0.05$) different. SD, Standard deviation; CV%, coefficient of variation percentage

These observations corroborated earlier reported trends of Glu and Asp being the most concentrated amino acids in some oil seeds [22], *Cola acuminata*, *Garcinia kola* and *Anacardium occidentale* [23] and some legumes even after different processing techniques [24] as well as raw *P. africana* [5]. Methionine (Met) was the least concentrated in the three samples of *P. africana* at 10.40, 4.20 and 13.10 mg/gcp respectively, while glycine (Gly) was the least concentrated in *R. communis* with values of 10.80, 4.00 and 16.8 mg/gcp respectively. The

observed low concentration of Met in these legume sample is in consonance with earlier reported least concentration of Met in some raw and processed legumes [24, 25]. As can be seen from Figure 1, *R. communis* has higher crude protein content than *P. africana*. Similarly, raw seeds of *R. communis* were richer (11 amino acids out of 17; 64.71%) than *P. africana* in the following amino acids; Lys, His, Arg, Thr, Val, Met, Ile, Leu, Phe, Asp and Ala. However, fermentation changed the trend making fermented *P. africana* to be apparently richer (12 amino acids out of 17; 70.58%) than fermented *R. communis* in all non-essential amino acids (except Asp) as well as Phe, Leu, Ile, Val and His. Whereas fermentation significantly ($p < 0.05$) increased amino acid compositions of the fermented seed sample of *P. africana* by 94.12%, it only, but significantly ($p < 0.05$), improved the amino acid compositions of *R. communis* by 52.94% over their respective raw samples. On the other hand, boiling significantly ($p < 0.05$) reduced the amino acid compositions by 47.06% in *P. africana* and 82.35% in *R. communis*. These reductive effects of boiling on protein and amino acid compositions of the seeds, as discussed earlier may be attributed to Amadori rearrangements that may go beyond the deoxy-ketosyl stage. It may also be due to formation of D-amino acids which results from high and prolonged heat treatment [21]. This was the case because, the method usually used for amino acid analysis will only detect L-amino acids from animal and plant proteins that do not produce racemisation [26].

Meanwhile, the higher crude protein contents observed in *R. communis* samples notwithstanding, the combined processes of boiling and fermentation improved amino acid quality of *P. africana* more. This may be attributed, partially to the observed higher reductive (82.35%) effect of boiling on the *R. communis* amino acid composition. Boiling being a pre-step before fermentation during local condiments production. Fermentation significantly ($p < 0.05$) improved all the amino acids except Asp in *P. africana*, and Arg, Thr, Val, Met, Ile, Leu, Phe and Glu in *R. communis*. The observed significant ($p < 0.05$) improvement in all essential amino acid compositions of *P. africana*, along with Lys and Met in *R. communis* corroborates earlier observed similar effect of fermentation on Lys, His, Arg, Thr, Val, Ile and Phe contents of cocoa nibs [27]. These observations followed the trend reported for 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 [28].

Tables 3 and 4 show various calculated parameters aimed at providing data on protein quality of the seed samples. The total amino acids (TAA) content of raw seeds of *P. africana* and *R. communis* were improved by the fermentation process from 733.00 mg/gcp and 823.00 mg/gcp to 962.60 mg/gcp and 894.10 mg/gcp respectively. These were close to or higher than those reported for fermented (708 mg/gcp) and unfermented (641 mg/gcp) cocoa nibs [27], *A. occidentale* (659 mg/gcp) [23] and dehulled samples of African yam bean (703-917 mg/gcp) [29]. The total non-essential amino acids (TNEAA) for the seed samples (mg/gcp) were: 422.00 (raw) and 558.10 (fermented) for *P. africana* (Table 3), and 422.40 (raw) and 463.80 (fermented) for *R. communis* (Table 4). The TNEAA values obtained were close to the value of 341-421 mg/gcp in processed and unprocessed cocoa nibs [27], 327-454 mg/gcp in Africa yam bean [29] and 337.6-356.2 mg/gcp in male fresh water crab [30]. The total essential amino acid (TEAA) compositions in mg/gcp of the raw and fermented seed samples of *P. africana* were 311.00 and 404.50, while those of *R. communis* were 401.10 and 430.30 respectively.

Table 3: Concentrations of essential, non-essential, acidic, basic, neutral, sulphur and aromatic amino acids (mg/g crude protein) of raw, boiled and fermented seed samples of *Prosopis africana*

| Amino acid groupings | Raw | Boiled | Fermen | Mean | SD | CV% |
|--|--------|--------|--------|--------|--------|-------|
| Total amino acid (TAA) | 733.00 | 637.40 | 962.60 | 777.70 | 167.10 | 21.49 |
| Total non-essential amino acid (TNEAA) | 422.00 | 370.70 | 558.10 | 450.30 | 96.84 | 21.51 |
| Total essential amino acid (TEAA) | | | | | | |
| with His | 311.00 | 266.70 | 404.50 | 327.40 | 70.35 | 21.49 |
| without His | 294.70 | 254.80 | 379.50 | 309.70 | 63.68 | 20.57 |
| % TNEAA | 57.57 | 58.16 | 57.98 | 57.90 | 0.30 | 0.52 |
| % TEAA | | | | | | |
| with His | 42.43 | 41.84 | 42.02 | 42.10 | 0.30 | 0.72 |
| without His | 40.20 | 39.97 | 39.42 | 39.86 | 0.40 | 1.01 |
| Total neutral amino acid (TNAA) | 435.10 | 378.20 | 577.40 | 463.60 | 102.60 | 22.13 |
| % TNAA | 59.36 | 59.33 | 59.98 | 59.56 | 0.37 | 0.62 |
| Total acidic amino acid (TAAA) | 199.40 | 182.70 | 264.00 | 215.40 | 42.94 | 19.94 |
| % TAAA | 27.20 | 28.66 | 27.43 | 27.76 | 0.79 | 2.83 |
| Total basic amino acid (TBAA) | 98.50 | 76.50 | 121.20 | 98.73 | 22.35 | 22.64 |
| % TBAA | 13.44 | 12.00 | 12.59 | 12.68 | 0.72 | 5.71 |
| Total sulphur amino acid (TSAA) | 31.60 | 24.70 | 55.00 | 37.10 | 15.88 | 42.81 |
| % TSAA | 4.31 | 3.88 | 5.71 | 4.63 | 0.96 | 20.65 |
| % Cys in TSAA | 67.09 | 83.00 | 76.18 | 75.42 | 7.98 | 10.58 |
| Total aromatic amino acid (TArAA) | 75.00 | 72.80 | 107.80 | 85.20 | 19.60 | 23.01 |
| % TArAA | 10.23 | 11.42 | 11.20 | 10.95 | 0.63 | 5.78 |
| P-PER | 24.54 | 23.57 | 31.36 | 26.49 | 4.25 | 16.03 |
| Leu/Ile ratio | 1.91 | 2.40 | 1.87 | 2.06 | 0.30 | 14.33 |
| Leu – Ile | 30.00 | 35.20 | 36.90 | 34.03 | 3.60 | 10.56 |
| % Leu - Ile | 4.09 | 5.52 | 3.83 | 4.48 | 0.91 | 20.31 |

SD, Standard deviation; CV%, coefficient of variation percentage

These were higher than 300 mg/gcp and 287 mg/gcp reported for unfermented and fermented cocoa nibs [27], and 298.2-327.8 mg/gcp of male fresh water crab [30]. In comparison with other common seeds, the TEAA we obtained, were lower than the levels (mg/gcp) in melon (534) and gourd oilseed (536) [22], and close to those of pigeon pea (452) [31], soybean (444) [32], cowpea (426) [33], and *Cajanus cajan* (436) [34]. The percent TNEAA ranged between 57.57% (raw) and 57.98% (fermented) for *P. africana* (Table 3); in *R. communis* (Table 4), it ranged from 51.29% (raw) to 51.87% (fermented); each with low CV% values of 0.52 and 0.92 respectively. The %TEAA (with His) ranged between 42.43% (raw) and 42.02% (fermented) in *P. africana* (Table 3), and between 48.71% (raw) and 48.13% (fermented) in *R. communis* (Table 4) with low CV% values of 0.72 and 0.98 respectively.

These results showed that the TNEAA and TEAA values of *P. africana* and *R. communis* were very close, and that fermentation did not alter so much, the overall seeds' percentage contents of TNEAA and TEAA, notwithstanding the observed individual increases in total

essential and non-essential amino acids composition after fermentation (Tables 1 and 2). The total neutral amino acids (TNAA) levels of the raw seeds of both plants were approximately the same (435 mg/gcp). However, fermentation increased the TNAA level of *P. africana* (577.4 mg/gcp) more than that of *R. communis* (467.7 mg/gcp).

Table 4: Concentrations of essential, non-essential, acidic, basic, neutral, sulphur and aromatic amino acids (mg/g crude protein) of raw, boiled and fermented seed samples of *Ricinus communis*

| Amino acid groupings | Raw | Boiled | Fermented | Mean | SD | CV% |
|--|--------|--------|-----------|--------|--------|-------|
| Total amino acid (TAA) | 823.50 | 688.40 | 894.10 | 802.00 | 104.50 | 13.03 |
| Total non-essential amino acid (TNEAA) | 422.40 | 350.60 | 463.80 | 412.30 | 57.28 | 13.89 |
| Total essential amino acid (TEAA) | | | | | | |
| with His | 401.10 | 337.80 | 430.30 | 389.70 | 47.29 | 12.13 |
| without His | 384.20 | 324.70 | 409.00 | 372.60 | 43.32 | 11.63 |
| % TNEAA | 51.29 | 50.93 | 51.87 | 51.36 | 0.47 | 0.92 |
| % TEAA | | | | | | |
| with His | 48.71 | 49.07 | 48.13 | 48.64 | 0.47 | 0.98 |
| without His | 46.65 | 47.17 | 45.74 | 46.52 | 0.72 | 1.56 |
| Total neutral amino acid (TNAA) | 435.70 | 346.10 | 467.70 | 416.50 | 63.03 | 15.13 |
| % TNAA | 52.91 | 50.28 | 52.31 | 51.83 | 1.38 | 2.66 |
| Total acidic amino acid (TAAA) | 254.10 | 234.70 | 273.60 | 254.10 | 19.45 | 7.65 |
| % TAAA | 30.86 | 34.09 | 30.60 | 31.85 | 1.94 | 6.10 |
| Total basic amino acid (TBAA) | 133.70 | 107.60 | 152.80 | 131.40 | 22.69 | 17.27 |
| % TBAA | 16.24 | 15.63 | 17.09 | 16.32 | 0.73 | 4.49 |
| Total sulphur amino acid (TSAA) | 45.10 | 33.90 | 53.00 | 44.00 | 9.60 | 21.81 |
| % TSAA | 5.48 | 4.92 | 5.93 | 5.44 | 0.51 | 9.30 |
| % Cys in TSAA | 38.80 | 30.97 | 42.28 | 37.35 | 5.79 | 15.51 |
| Total aromatic amino acid (TArAA) | 78.20 | 69.20 | 83.00 | 76.80 | 7.01 | 9.12 |
| % TArAA | 9.50 | 10.05 | 9.28 | 9.61 | 0.40 | 4.13 |
| P-PER | 26.11 | 24.06 | 26.86 | 25.68 | 1.45 | 5.64 |
| Leu/Ile ratio | 1.74 | 2.27 | 1.75 | 1.92 | 0.30 | 15.79 |
| Leu – Ile | 27.50 | 33.60 | 28.50 | 29.87 | 3.27 | 10.95 |
| % Leu - Ile | 3.34 | 4.88 | 3.19 | 3.80 | 0.94 | 24.60 |

SD, Standard deviation; CV%, coefficient of variation percentage

Many plant proteins, especially the legumes, contain substantially more Cys than Met [24]. This assertion agrees with our observation in *P. africana* in which total sulphur amino acid (TSAA) for the raw and fermented seed samples were 31.60 and 55.0 mg/gcp, with the percentage of Cys in the TSAA (%Cys/TSAA) being 67.09% and 76.18% respectively. However, the %Cys/TSAA in raw and fermented *R. communis* samples were low at 38.80% and 42.20% respectively. Thus, while the %Cys/TSAA of *P. africana* followed the pattern of 62.9% in coconut endosperm [35], and 58.9% (raw), 72.0% (steeped) and 71.1% (germinated) guinea corn [28], those of *R. communis* followed the trend in *C. acuminata* (44.3%) [23], *G. kola* (37.8%), and fermented (46.3%) and unfermented (44.1%) cocoa nibs [27], as well as animal

proteins; *Macrotermes bellicosus* (36.3%) [36], *Archatina archatina* (38.8%) and *Archachatina marginata* (35.5%) [37]. Thus, our result of %Cys/TSAA in *P. africana* corroborated the statement that Cys may supply up to one-third of the need for TSAA [13].

Muller and Tobin [21] reported that experimentally determined protein efficiency ratio (PER) usually ranged from 0.0 for a very poor protein to a maximum of just over 4. Our results of predicted PER (P-PER) showed that fermented samples of *P. africana* (2.72) and *R. communis* (2.26) would likely be utilized more in the body than those of raw (2.03 and 2.19) or boiled (1.94 and 1.99) samples respectively. The P-PER results of the fermented samples compared favourably with those of processed common legumes (2.15 – 2.95), fermented cocoa nibs (2.55), and male fresh water crab (2.70) [24,27,30].

The Leu/Ile ratios of the raw, boiled and fermented samples were 1.91, 2.40 and 1.87 mg/gcp for *P. africana*, and 1.74, 2.27 and 1.75 mg/gcp for *R. communis* respectively. The increase in Leu/Ile ratio we observed after boiling is a consequence of the significant ($p < 0.05$) decrease in Ile contents (see Tables 1 and 2) of both seed samples, instead of an increase in Leu content. Meanwhile, high Leu content in diet impairs tryptophan and niacin metabolism, might be a factor in pellagra development, and niacin deficiency in sorghum consumers [38,39]. Thus, it was suggested that Leu/Ile balance is a more important factor than dietary excess of Leu alone in regulating the metabolism of Trp and niacin, and hence the disease process [27]. Furthermore, it has been reported that animals fed sorghum proteins containing less than 110 mg/gcp of Leu did not suffer from nicotinic acid deficiency [40]. Interestingly, none of the plants' samples we studied had Leu levels up to this critical level.

Tables 5 and 6 show the amino acid scores (AAS), based on the provisional amino acids, for the samples of *P. africana* and *R. communis* respectively. In Table 5, Thr was the limiting amino acid for raw (0.62), boiled (0.54) and fermented (0.69) samples of *P. africana*.

Table 5: Essential amino acid scores of raw, boiled and fermented seed samples of *Prosopis Africana*

| Amino acid | Raw | Boiled | Fermented | Mean | SD | CV% |
|------------|------|--------|-----------|------|------|-------|
| Ile | 0.82 | 0.63 | 1.07 | 0.84 | 0.22 | 26.27 |
| Leu | 0.90 | 0.86 | 1.14 | 0.97 | 0.15 | 15.67 |
| Lys | 0.74 | 0.63 | 0.86 | 0.74 | 0.12 | 15.48 |
| Met + Cys | 0.90 | 0.71 | 1.57 | 1.06 | 0.45 | 42.62 |
| Phe + Tyr | 1.25 | 1.21 | 1.80 | 1.42 | 0.33 | 23.22 |
| Thr | 0.62 | 0.54 | 0.69 | 0.62 | 0.08 | 12.17 |
| Try | - | - | - | - | - | - |
| Val | 0.81 | 0.76 | 1.06 | 0.88 | 0.16 | 18.33 |

SD, Standard deviation; CV%, coefficient of variation percentage

Table 6: Essential amino acid scores of raw, boiled and fermented seed samples of *Ricinus communis*

| Amino acid | Raw | Boiled | Fermented | Mean | SD | CV% |
|------------|------|--------|-----------|------|------|-------|
| Ile | 0.93 | 0.66 | 0.95 | 0.84 | 0.16 | 19.13 |

| | | | | | | |
|-----------|------|------|------|------|------|-------|
| Leu | 0.92 | 0.86 | 0.95 | 0.91 | 0.05 | 5.04 |
| Lys | 0.80 | 0.75 | 0.96 | 0.84 | 0.11 | 13.11 |
| Met + Cys | 1.29 | 0.97 | 1.51 | 1.26 | 0.27 | 21.61 |
| Phe + Tyr | 1.30 | 1.15 | 1.38 | 1.28 | 0.12 | 9.15 |
| Thr | 0.93 | 0.91 | 0.96 | 0.93 | 0.03 | 2.70 |
| Try | - | - | - | - | - | - |
| Val | 0.97 | 0.81 | 1.00 | 0.93 | 0.10 | 11.02 |

SD, Standard deviation; CV%, coefficient of variation percentage

These observations are in agreement with a previous report of Thr at an AAS of 0.55 being the limiting amino acid in raw seeds of *P. africana* [5]. To fulfill the day's need for all the EAA using *P. africana* seeds, 100/62 or 1.61 times as much raw seed protein, 100/54 or 1.85 times as much boiled seed protein and 100/69 or 1.45 times of fermented seed protein, would have to be eaten when they are the sole protein in the diet. In Table 6, Lys was the limiting amino acid in raw seeds (0.80), and Ile or Leu in fermented seeds (0.95) of *R. communis*. Their correction values would be 1.25 times the protein in raw seeds and 1.05 times in fermented seeds. The entire CV% for the samples of both *P. africana* (Table 5) and *R. communis* (Table 6) were generally low.

CONCLUSION:

P. africana and *R. communis* are good sources of protein and contain both essential and non-essential amino acids in quantities comparable with some animal proteins and other common legumes. While fermentation elicited about 31% and 9% overall positive buildup in total amino acid content in *P. africana* and *R. communis*, boiling caused 13% and 16% destruction of amino acids in the seeds. Thus, they can serve well in complementing weaning food for children in the tropics where protein malnutrition is one of the major causes of child morbidity and mortality.

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