ASSESSMENT OF THE NUTRITIVE AND ANTI-NUTRITIVE COMPOSITIONS OF FERMENTED AND UNFERMENTED AFRICAN CUSTARD APPLE (*Annona senegalensis*) SEEDS FROM NIGER STATE, NIGERIA

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Abstract: The assessment of nutritive and anti-nutritive compositions of fermented and unfermented seeds of *Annona senegalensis* from Niger State, Nigeria was carried out using standard analytical methods. The proximate parameters determined were moisture, protein, ash, fat and fibre of the unfermented seed were 18.07±0.03, 16.90±0.14, 3.62±0.23, 27.54±0.45 and 7.34±0.11%, respectively; while the moisture, protein, ash, fat and fibre of the fermented seed for 24 and 48 h were 21.00±0.51 and 22.78±0.21, 18.42±0.32 and 25.89±0.05, 4.00±0.50 and 4.22±0.32, 23.00±0.10 and 21.76±0.31, 5.35±0.16 and 4.05±0.14%. The carbohydrate content was lowest in the seed fermented for 24 h (21.30±0.46%) and highest in the unfermented seed (26.60±0.54%). Energy values obtained in this work were 1757.29±0.33, 1616.34±0.14 and 1573.35±0.52 Kcal/100g for the unfermented, 24 and 48 h fermented samples, respectively. The fermentation generally improved the mineral contents but decreased the anti-nutrient contents of the seeds. The amino acid profiles makes the studied seed protein of high nutritive. Thus, large-scale production of fermented *A. senegalensis* seeds will be a valuable source of nutrients for humans and animals.

Keywords: *Annona senegalensis*, fermented, unfermented, nutrient, anti-nutrient

Introduction

In northern Nigeria, malnutrition and vitamins deficiency are quite common among infants and children. This is largely manifested in their greater susceptibility to infections and poor nutrition. Vitamins deficiency impairs growth and development as a result of their involvement in metabolic functions (Franzo *et al.*, 2013). Fruits and vegetables are low in fat, salt and sugar but are good sources of dietary fibre. Thus, they are a part of a well-balanced regular diet and a healthy, active lifestyle. A high intake of fruits and vegetables has been found to reduce obesity, maintain a healthy weight, lower cholesterol, lower blood pressure, reduce the risks of colon cancer and other cancers (Bello, 2014). Fruits are excellent sources of healthy phytochemical, antioxidants, and fibre too. Therefore, taking high contents of catechins and green tea for examples may protects against death from all causes, especially cardiovascular diseases (Ebert, 2014).

African custard apple plant is a wild growing shrub or small tree up to 7 meters or more in height. It is not a resilient plant in nature but has been cultivated for its leaves, fruits, flowers, barks and stems for medicinal purposes. Its leaves are alternate, simple, oblong to oval to almost ovoid, 6 - 185 x 25 - 120 mm in size. The stems are different in their natural colour which is grey and smooth but coarse in older trees. The undeveloped branches are with yellow hairs which are lost during development. The flowers are up to 35 mm in diameter while the stalk is up to 30 mm long, which is directly above the leaf axils. The fruit is formed from several fused, fresh and ovate carpels about 45 mm in diameter. At early development, it is dark green ripening to yellow and finally orange during the developmental stage of its life. It has a curved inner whorl over the stamens and ovary and several stamen (Coates, 2002). This plant is common and quite wild in the shrubbery, open bush and along rivers and streams in Nigeria. It is found throughout Northern Nigeria, primarily in the Nasarawa, Kaduna, Kano, Plateau and Niger States and Federal Capital Territory (FCT), Abuja (Alqasim, 2013). Although many of these plants have been identified, scanty data are available on their chemical composition for the prospect of their utilization despite the fact that some could have good nutritional application (FMH, 2016). The objective of this study was to determine the effect of fermentation on the nutritients and anti-nutritient contents of the seed of African custard apple (*A. senegalensis*) seeds.

Materials and Methods

Sample preparation

The sample was collected between July and September, 2016. The seeds were separated, washed, rinsed with clean water and dried at room temperature for some days. After drying, they were ground into fine powder with porcelain mortar and pestle; sieved with mesh size of 0.5 mm. The traditional methods of African locust beans fermentation was adopted in this work with modification. 200 g of African custard apple (*A. senegalensis*) seeds powder was weighed into 1000 cm^3 conical flask, 50 cm^3 of distilled water was added while 0.5 g of commercial baker's yeast (*Saccharomyces cerevisiae*) was added to the mixture. It was stirred, covered and allowed to ferment for 24 h. The same process was repeated for the fermentation at 48 h. The resultant mixture was dried in a freeze dryer and kept for further analysis.

Methods

Proximate analyses

The moisture, ash, fat and protein contents of the *Annona senegalensis* seed flour were determined out as described by the AOAC (2006). Total carbohydrate content was determined by subtracting percentage moisture, ash, crude fibre, protein, and fat from 100%. The energy value (kcal/100g) was estimated by multiplying the percentage of crude protein, crude lipid and carbohydrate by 4, 9 and 4 respectively as conversion factors (AOAC, 2006).

Mineral analysis

The sample was digested by weighing in triplicate 1.00 g into beakers and 10 cm^3 of the acid mixture ($HClO_4$:H_2SO_4:HNO_3) in the ratio of 1:4:3 was added in each case. The mixture was swirled and left in a fume cupboard overnight. The samples were then digested on a Kjedhal digestion block until the solutions became quite clear. The digests were allowed to cool, diluted with 20 cm^3 of water, filtered using Whatman filter papers, made up to mark with deionized water in 100 cm^3 volumetric flasks and then transferred into sample bottles. The samples were analyzed for their mineral contents (Ca, Cu, Fe, Zn, Mn and Mg) using atomic absorption spectrophotometer (AAS) Buck model 210 VGP. A flame photometer (AA-500F, China) was used for the determination of potassium and sodium, while phosphorus was determined colorimetrically using the vanudo-molybodate colorimetric method (KF1700, Sweden) (AOAC, 2006).

Determination of amino acid profile

The sample was defatted using chloroform/methanol mixture in 2:1 proportions. About 4.00 g of the sample was kept in extraction thimbles and extracted for 15 h in Soxhlet extractor (Nieman *et al.,* 1992).

Nitrogen determination

In each case, 200 mg of the powdered sample was weighed, wrapped in Whatman filter paper (No.1) and kept in the Kjeldhal flask. 0.50 g of sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) at 10:5:1 ratio was added into the digestion flask. 10 cm^3 of concentrated sulphuric acid and anti-bumping agents were also added to the mixture. The distillate was then titrated with standardizing 0.01 $moldm^{-3}$ hydrochloric acid.

$$\text{Percentage Nitrogen} = \frac{(a-b)\times 0.01\times 14\times V\times 100}{W\times C} \quad (1)$$

Where: a = titre value of the digested sample; b = titre value of the blank sample; V = volume after dilution (100 cm^3); W = weight of dried sample (mg); C = aliquot of the sample used (10 cm^3)

About 2.00 g of the dried sample was weighed into extraction thimble and defatted with 2:1 chloroform and methanol mixture using Soxhlet extractor. From the defatted sample, 1.0 g was weighed into glass ampoule and 7 cm^3 of 6.0 $moldm^{-3}$HCl was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was sealed and placed in an oven preset at 105±5°C for 22 h. The ampoule was allowed to cool before breaking open at the tip and the content filtered. The filtrate was then evaporated to dryness at 40°C and the residue was dissolved in 5 cm^3 acetate buffer (pH 2.0) and stored in plastic specimen bottles. About 5–10 µl was dispensed into the cartridge of the sequential multi-sample amino acid analyzer (TSM). The net height of each peak produced by the chart recorder of TSM was measured. The half-height of the peak on the chart was found and the width of the peak on the half height was accurately measured and recorded. Approximately area of each peak was then obtained by multiplying the height by the width at half-height.

The norcleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE = \frac{\text{Area of Norceucine Peak}}{\text{Area of each amino acid}} \quad (2)$$

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

Where $S_{std} = NE_{std} \times \text{Molecular weight} \times \mu MAA_{std}$ (3)

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

Concentration (g/100g protein) = NH x W@NH/2 x S_{std} x C (4)

$$C = \frac{\text{Dilution factor}\times 16}{\text{Sample weight (g)}\times\%N\times 10\times\text{vol loaded}} \div Nh \times W(\text{norleucine}) \quad (5)$$

Where Nh = Net height, W = Width at half height and W(norleucine) = Width of norleucine.

Evaluation of anti-nutritional factors

The phytate, cyanide and oxalate contents were determined using the methods of (AOAC, 2006).

Statistical analysis

All determinations were performed in triplicate. The triplicate values of analytes obtained in this study were analyzed using one way analysis of variance with Duncan Multiple Range test at 95% confidence or P≤0.05.

Results and Discussion

The proximate composition (%) and energy contents (kcal/100g) of fermented and unfermented seeds flour of African custard apple are presented in Table 1. The moisture contents ranged from 18.07±0.03 (unfermented) to 22.78±0.21% (fermented at 48 h). The result obtained from this work was higher than that of pigeon pea flour (7.44±0.04%) reported by Babalola and Giwa (2012). The values obtained for samples fermented of both 24 and 48 h in this work, were lower than the respective 61.50±2.12 and 52.50±3.54% reported for soyabean seeds flour by Babalola and Giwa (2012). The moisture contents increased with the fermentation with period probably due to increase in the relative humidity. The shelf life of the fermented samples on the basis of their moisture contents would be lower than those of the raw samples. This is because, high moisture content speed up deterioration since excess water aids microbial activities (Ogunyinka *et al.,* 2017). The mean crude fibre of the seed ranged from 4.23±0.16 (unfermented) to 5.04±0.15% (fermented for 48 h). The crude fibre contents in the present work were low compared to the range of 4.05±0.14 to 7.34±0.11% reported for the raw and fermented soya beans reported by Babalola and Giwa (2012). The fibre contents of the three samples decreased by fermentation as a result of the activities of extracellular enzymes which converted fibres to soluble carbohydrates (Jolaoso *et al.,* 2014). The decrease in crude fibre contents was in agreement with the trend (20.08±0.11% for raw) and 18.33±0.17% for fermented soybean seeds reported by Babalola and Giwa (2012); although the values in this work, were low relative to those of these authors. The low fibre contents will be of advantage to maintain the health of the gastrointestinal tract although excess fibre may bind trace elements, like zinc, copper and iron leading to deficiency (Mosisa, 2017). The mean carbohydrate contents ranged from 21.30±0.46 (fermented for 48 h) to 26.60±0.54% (unfermented).

The result obtained for the raw and fermented samples for 24 and 48 h as reported for pigeon pea flour by Mbaeyi-Nwaoha and Obetta (2016) were lower than the 54.70±0.11, 52.27±0.01 and 49.82±0.42%, respectively obtained in the presents study. The crude fat contents of the samples in the present work ranged from 4.02±0.23 (fermented for 48 h) to 7.00±0.06 % (raw seeds). The decrease in crude fat contents of the samples might be attributed to the increase in the activities of the lipolytic enzymes during fermentation which may have hydrolysed fat components into fatty acids and glycerol (Adebowale and Maliki, 2011). The decrease in crude fat was in disagreement with increased values (19 to 23%) for fermented soybeans reported by Thingom and Chhetry (2011). The crude protein values of the samples ranged from 16.90±0.14 (unfermented) to 25.89±0.05 (fermented for 48 h). The increase in protein contents of the plant seeds is in agreement with the result of soybeans seeds reported by Babalola and Giwa (2012). Although, their values were lower compared to those of the present study but in agreement with the value 25% reported for soybeans seeds fermented for 48 h reported by Thingom and Chhtry (2011). The ash contents ranged from 3.62±0.23 (unfermented) to 4.22±0.32% (fermented for 48 h). This result indicates that the ash contents of the samples increased during fermentation which might be attributed to the increase in moisture content. Similar increase was observed by Babalola and Giwa (2012) during the fermentation of soybean flour (3.31±0.44 to 5.38±0.22%). However, the values obtained in this study were lower than the 6.21±0.05 to 6.91±0.53% reported for locust beans seeds by Aremu *et al.* (2015).

Table 1: Proximate compositions (%) and energy contents (kcal/100g) of the Seeds flour of African custard apple

Parameters	Unfermented	Fermented for 24 h	Fermented for 48 h
Moisture	18.07±0.03[a]	21.00±0.51[b]	22.78±0.21[c]
Crude Protein	16.90±0.14[a]	18.42±0.32[b]	25.89±0.05[c]
Ash	3.62±0.23[a]	4.00±0.50[b]	4.22±0.32[c]
Crude Fibre	7.34±0.11[c]	5.35±0.16[b]	4.05±0.14[a]
Crude Fat	27.54±0.45[c]	23.00±0.10[b]	21.76±0.31[a]
Carbohydrate	26.53±0.35[ab]	26.60±0.54[bc]	21.30±0.46[a]
Calories	1757.29±0.33[c]	1616.34±0.14[b]	1573.35±0.52[a]

Mean within a row with same superscripts were not significantly different at (p≥0.05)

Table 2: Mineral compositions of African custard apple (mg/100g)

Parameters	Unfermented	Fermented for 24 h	Fermented for 48 h
Na	126.13±0.15[a]	129.50±0.82[b]	130.96±0.11[c]
K	160.29±0.80[a]	170.32±0.50[b]	172.42±0.42[c]
P	105.60±0.26[a]	111.43±0.39[b]	114.27±0.51[c]
Ca	7.05±0.66[a]	9.22±0.36[b]	11.02±0.40[c]
Fe	15.30±0.45[c]	12.01±0.52[b]	10.63±0.12[a]
Mg	11.30±0.26[c]	8.11±0.41[b]	7.02±0.20[a]
Zn	5.33±0.21[a]	6.98±0.62[b]	8.07±0.55[c]
Cu	9.55±0.13[c]	5.20±0.60[b]	4.30±0.16[a]
Mn	3.35±0.24[b]	2.97±0.43[a]	5.17±0.34[c]

Mean within a row with same superscripts were not significantly different at (p≥0.05)

The mineral contents (mg/100g) of fermented and unfermented of African custard apple seed flours are presented in Table 2. Calcium is an essential mineral for bone development (Mathew *et al*. 2014). The calcium concents of the flours ranged from 7.05±0.66 (unfermented) to 11.02±0.40 mg/100g (fermented for 48 h). These values were higher than the 3.05±0.30 mg/100g (unfermented) to 8.71±0.09 mg/100g (fermented for 48 h) range reported for pigeon pea flour by Mbaeyi-Nwaoha and Obetta (2016). However, the values were lower than the 28.20 mg/100g for seeds fermented for 48 h reported for African oil beans seed by Balogun (2013). The concentrations of iron in the samples which ranged from 10.63±0.12 (fermented for 48 h) to 15.30±0.45 mg/100g (raw samples) were higher than the 6.13±0.50 mg/100g reported for sesame seed flour by Makinde *et al*. (2013). Although, the values were lower than the 29.91±0.01 mg/100g for African yam bean reported by Adamu *et al*. (2015). Thus, this plant seeds would provide adequate iron needed by humans. The contents of magnesium ranged from 7.02±0.20 (fermented for 48 h) to 11.30±0.26 mg/100g (unfermented plant seed). There was gradual decrease in the magnesium contents of the seed flour as the fermentation period increased. This may be as a result of leaching that took place during the fermentation. However, the magnesium contents of the samples in this study are higher than those for fermented soybeans (4.69±0.01 mg/100g) reported by Omodara and Olowomofe (2015). On the other hand, they were lower than the 60.50±0.28 mg/100g and 186.05±0.02 mg/100g reported for beans and mug beans by Adamu *et al*. (2015). The manganese contents of the flours ranged from 2.97±0.43 (unfermented) to 5.17±0.34 mg/100g (fermented for 48 h). These values were high when compared to the 0.2±0.01 mg/100g reported for fermented African locust bean seeds by Abdulrahman *et al*. (2016). However, the values were low when compared with the 10.00±0.01 mg/100g reported for African yam beans by Adamu *et al*. (2015). The mean potassium contents ranged from 160.29±0.80 (unfermented) to 172.42±0.42 mg/100g (fermented for 48 h). These values were lower than the 1205.46±0.16 mg/100g reported for *J. cathartica* seeds fermented for 48 h by Oladele and Oshodi (2008) but higher than 76.80±0.22 mg/100g reported for pigeon pea flour reported by Mbaeyi-Nwaoha and Obetta, (2016). Potassium plays important role in the human body and sufficient amounts of it in the diet protect against heart disease, hypoglycaemia, diabetes, obesity and kidney dysfunction (Mathew *et al*., 2014).

Adequate intake of this mineral from the diets has been found to lower blood pressure by antagonizing the biological effects of sodium (Mathew *et al.*, 2014). The mean copper concentrations of the samples ranged from 4.30±0.16 (fermented for 48 h) to 9.55±0.13 mg/100g (raw). The copper contents were higher than 0.90±0.00 mg/100g and 0.46±0.00 mg/100g reported for beans and soybean respectively by Adamu *et al*. (2015). This might have been as a result of the breaking down of some complex metallic compounds in the sample during fermentation. The mean zinc contents of the samples ranged from 5.33±0.21 (unfermented) to 8.07±0.55 mg/100g (fermented for 48 h). These were higher than 5.81 mg/100g (unfermented) to 6.38 mg/100g (fermented for 48 h) range for *C. altissimum* seed reported by Jolaoso *et al.* (2014) except for the value for the unfermented samples.

However, the values were low compared to 18.33±0.01 mg/100g reported for mug beans by Adamu *et al*. (2015). The phosphorus contents of the samples ranged from 105.60±0.26 (unfermented) to 114.27±0.51 mg/100g (fermented for 48 h). This increase compared well with the result of Jolaoso *et al*. (2014) who reported an increase in the level of phosphorus during the fermentation of *C. attissimum* which ranged from 560 mg/100g to 720 mg/100g. The phosphorus content of the fermented seeds in the present study is desirable since the element is needed in the formation of strong bones and teeth. It also plays a role in energy metabolism of the cells (Jolaoso *et al*., 2014).

Table 3: Amino acid profile of the samples (g/100g Protein)

Parameters	Unfermented	Fermented for 24 h	Fermented for 48 h
*Leucine	9.57±0.08[a]	10.66±0.09[b]	10.52±0.05[b]
*Lysine	4.35±0.04[a]	5.02±0.10[b]	4.97±0.05[ab]
*Isoleucine	3.21±0.09[a]	3.89±0.05[b]	3.60±0.09[ab]
*phenylalanine	3.72±0.08[a]	4.63±0.07[b]	4.26±0.08[b]
*Tryptophan	1.05±0.02[a]	1.09±0.07[a]	1.06±0.02[a]
*Valine	3.68±0.11[a]	4.76±0.09[b]	4.60±0.10[b]
*Methionine	1.23±0.09[a]	1.96±0.07[b]	1.80±0.04[b]
*Histidine	2.17±0.10[a]	3.51±0.06[b]	3.58±0.07[b]
*Threonine	3.33±0.06[a]	3.80±0.08[b]	3.62±0.02[b]
“Cysteine	1.09±0.06[a]	2.00±0.05[b]	1.92±0.02[b]
“Proline	3.65±0.09[a]	4.20±0.02[b]	4.00±0.05[b]
“Arginine	5.68±0.02[a]	6.10±0.08[b]	6.02±0.06[b]
“Tyrosine	3.53±0.03[a]	3.94±0.02[b]	3.72±0.04[b]
“Glysine	3.99±0.02[a]	4.37±0.07[b]	4.40±0.08[b]
Serine	3.83±0.06[a]	4.25±0.02[bc]	4.20±0.06[b]
Aspartic acid	9.40±0.05[a]	9.86±0.04[b]	9.71±0.05[ab]
Glutamic acid	11.96±0.08[a]	13.53±0.08[b]	13.39±0.03[b]
Alanine	4.70±0.06[a]	5.32±0.09[b]	5.30±0.07[b]
%TEAA	40.31±0.03[a]	42.69±0.08[c]	41.92±0.06[b]
%TCEAA	22.38±0.10[b]	21.47±0.10[a]	22.12±0.02[b]
%TNEAA	37.29±0.11[c]	34.33±0.09[a]	35.95±0.05[b]

Mean within a row with same superscripts were not significantly different at (p≥0.05); Represent essential amino acids and “represent conditionally amino acids, TEAA: Total essential amino acids, TCEAA: Total conditionally essential amino acids, TNEAA: Total non-essential amino acids.

The amino acid compositions (g/100g protein) of the fermented and raw seed sample are presented in Table 3. The physiological role of dietary proteins is the provision of substrates required for the synthesis of body proteins and other metabolically important nitrogen-containing compounds. Therefore, the contents of the nutritionally indispensable amino acids in food proteins are usually the primary determinants of nutritional quality of proteins

(Mohanty *et al.*, 2014). Leucine is the only dietary amino acid that stimulates muscle protein synthesis and has important therapeutic role in stress conditions like burn, sepsis and trauma (Wu, 2010). Leucine is an essential amino acid, in this present study it had concentrations that ranged from 9.57±0.08 (unfermented) to 10.66±0.09 (fermented for 24 h) g/100g protein. There were increases in the leucine contents of samples as the period of fermentation increased. The increases might have been as a result of the formation of intermediate compounds which during metabolism, reacted with ammonia and were converted to a amino acids which in turn were useful in the formation of other amino acids (Okechukwu *et al.*, 2012). These values were high when compared with the 2.89±0.05 g/100g protein of *Astyanax fasciatus* leucine content reported by Furuya *et al*. (2015). Also, Buang and Taib (2014) recorded increase in leucine values during the fermentation of groundnut [1.74±0.04 g/100g protein (fermented for 18 h)] to 2.55±0.01 g/100g protein (for 30 h fermentation), although the values reported were lower than the values for the present study. These values for this study were however, lower than the 64.50±2.01 (raw) to 66.50±2.33 g/100g (fermented for 48 h) for *Ricinus communis* seed reported by Igwe *et al*. (2012). Therefore, leucine in the plant seeds can greatly contribute to the nutritional requirements of man and his animals.

Lysine is an essential amino acid extensively required for optimal growth and its deficiency leads to immunodeficiency. It is also used for preventing and treating cold sores (Wu, 2013). The lysine contents ranged from 4.35±0.04 (raw) to 5.02±0.10 g/100g protein (fermented for 24 h). Prolonged fermentation up to 48 h showed significant decrease in the lysine contents of the samples. These values were however higher than the 0.99±0.13 g/100g protein (raw) to 1.64±0.01 g/100g protein (fermented for 30 h) range reported for garbanzo beans by Bujang and Taib (2014). However, the lysine contents were low compared to the 40.60±1.10 (raw) to 47.20±0.30 g/100g (fermented for 72 h) range reported for *P. africana* by Igwe *et al*. (2012).

Isoleucine is a branched chain amino acid needed for muscle formation and proper growth. Chronic renal failure patients on haemodialysis have low plasma level of the branched chain amino acids such as leucine, valine and isoleucine (Monirujjaman and Ferdouse, 2014). The contents of isoleucine ranged from 3.21±0.09 (unfermented) to 3.89±0.05 g/100g protein (fermented for 24 h). There were significant decreases as fermentation reached 48 h in the concentrations of isoleucine which might have been as a result of reactions of intermediate compounds formed during metabolism with ammonia and were thus, converted to amino acids which, in turn, were useful in the formation of other amino acids (Okechukwu *et al.*, 2012). The concentrations of this amino acid in the samples of the present study were high when compared with the 0.84±0.05 g/100g protein (raw) to 1.28±0.05 g/100g protein (fermented for 48 h) reported for groundnut seeds by Bujang and Taib (2014). Although, the values were lower than 5.41 g/100g protein reported for African oil beans by Okechukwu *et al*. (2012). Also, the values were lower than the 10.40 g/100g protein isoleucine content reported for *Stolephorus waitei* by Mohanty *et al*. (2014).

The phenylalanine contents ranged from 3.72±0.08 (unfermented) to 4.63±0.07 g/100g protein (fermented for 24 h). Extension of fermentation period up 48 hours showed significant decreases in the phenylalanine contents of the samples. The phenylalanine contents of the samples in this work were higher than 1.78±0.02 g/100g protein to 2.17±0.03 g/100g protein for the unfermented and fermented samples reported for garbanzo beans by Bujang and Taib (2014). Also, Okechukwu *et al.*, (2012) reported 3.03 g/100g protein (raw) to 2.62 g/100g protein (fermented for 48 h) phenylalanine contents for African oil beans which are lower than the values obtained in this study. However, these values were lower than the 6.30±0.20 g/100g protein reported for *Anabas testudineus* by Mohanty *et al.* (2014). In addition, 41.60±3.01 (raw) to 67.20±1.21 g/100g protein (fermented for 72 h) range was reported for *Prosopis africana* by Igwe *et al.* (2012) which are higher than the range obtained in this study.

Tryptophan which is a precursor for serotonin, melatonin and tryptamine is a brain neurotransmitter theorized to oppress pain (Richard *et al.* 2009). Free tryptophan enters the brain cells to form serotonin. Thus tryptophan supplementation has been used to increase serotonin production in attempt to increase tolerance to pain (Richard *et al.* 2009). The tryptophan contents obtained ranged from 1.05±0.02 (unfermented) to 1.09±0.07 g/100g protein (fermented for 24). These values were higher than 0.65 g/100g dietary protein reported for *A. fasciatus* by Furuya *et al.* (2015). These were however, lower than the 2.10±0.50 g/100g protein reported for *Stolephorus waitei* by Mohanty *et al.* (2014). Methionine is used for treating liver disorders, improving wound healing and treating depression, alcoholism, asthma, radiation side effects, allergies, copper poisoning, drug withdrawal, Parkinson's disease and schizopherenia (Mohanty *et al.*, 2014). The contents of methionine ranged from 1.23±0.09 (nfermented) to 1.96±0.07 g/100g protein (fermented for 24 h). The increase in the methionine concentrations during the fermentation period might have been as a result of formation of intermediate compounds during metabolism which might have reacted with ammonia and converted to amino acids which in turn were useful in the formation of other amino acids (Okechukwu *et al.*, 2012). These values obtained for the samples were low compared to reported value for *P. africana* (10.40±0.10 to 13.10±010 g/100g protein for unfermented and fermented for 48 h) by Igwe *et al.* (2012). These were however, higher than the 1.04±0.01 g/100g protein methionine reported *Astyanax fasciatus* by Furuya *et al.* (2015). Valine is needed for muscle metabolism tissue repair and the maintenance of proper nitrogen balance in the body. It is helpful in treating liver and gall bladder disorders, and it is good for correcting the type of severe amino acid deficiencies caused by drug addiction (Wu, 2013). The concentrations of valine in the samples ranged from 3.68±0.11 g/100g protein (unfermented) to 4.76±0.09 g/100g protein (fermented for 24 h). The values obtained in this study were lower than the 5.36 (raw) to 6.89 g/100g protein (fermented for 48 h) valine level reported for African oil beans by Okechukwu *et al.* (2012). However, the values were high compared to the 1.00±0.13 (unfermented) to 1.58±0.06 g/100g protein (fermented for 30 h) valine values reported for groundnut by Bujang and Taib (2014). They were however, low when compared with the 40.30±0.80 (raw) to 53.20±0.60 g/100g protein (fermented for 72 h) *P. africana* seeds reported by Igwe *et al.* (2012). Histidine plays important roles in protein interaction and is also a precursor of histamine. It is also needed for growth and repair of tissue, for maintenance of the myelin sheaths and in removing heavy metals from the body (Liao *et al.*, 2013). The histidine concentrations in the samples ranged from 2.17±0.10 (unfermented) to 3.58±0.07 g/100g protein (fermented for 48 h). Fermentation for 48 hours showed significant decrease ($p<0.05$) in this amino acid in these plant seeds. These values were however, higher than the 0.79±0.01 (unfermented) to 1.29±0.02 g/100g protein (fermented for 30 h) reported for the garbanzo beans by Buang and Taib (2014). On other hand, they were, lower than the 7.90±0.60 g/100g protein reported for *Rastrelliger kanagurta* by Mohanty *et al*. (2014). In a similar observation, Okechukwu *et al*. (2012) recorded decreased histidine concentrations after the fermentation of African oil beans (1.81 for the raw to 1.43 g/100g protein for fermented for 48 h). This amino acid is very important for the growing and development of infants, therefore incorporation of these plant seeds into infant foods would enhance the growth and development of children particularly in developing nations where animal based-complementary foods are expensive.

Threonine is used for treating various nervous system disorders including spinal plasticity, multiple sclerosis, familia, spastic paraparesis and amyotrophic lateral sclerosis (Costa *et al.*, 2010). The contents of threonine obtained in this work ranged from 3.33±0.06 (unfermented) to 3.80±0.08 g/100g protein (fermented for 24 h). Fermentation for 48 hours significantly ($p<0.05$) decreased this amino acid in the seeds. The values of threonine in these samples were higher than the 0.73±0.03 (unfermented) to 1.09±0.03 g/100g protein (fermented for 30 h) range reported for groundnut by Bujang and Taib (2014). Also, the values were higher than the 2.54±0.01 (unfermented) to 3.06±0.02 g/100g protein (fermented for 48 h) range reported for African locust beans flour by Ijarotimi and Keshinro (2012). The % total essential amino acids (%TEAAs) ranged from 40.31±0.03 for unfermented to 42.69±0.08 g/100g protein in fermented for 24 h. The TEAAs obtained in this study for the samples were higher than the TEAANs of groundnut (7.80±0.60 for unfermented to 12.29±0.11 g/100g protein for fermentation at 30 hours) reported by Buang and Taib (2014). However, the value was lower when compared with the 59.98 % reported for fermented *P. Africana* seeds by Igwe *et al.* (2012).

Conditionally essential amino acids are those whose synthesis may be limited under certain pathophysiological conditions, such as infant prematurity or severe catabolic stress which may occur with surgery or trauma (Ripps and Shen, 2012). Glycine plays an important role in metabolic regulation, preventing tissue injury, enhancing anti-antioxidant activity, promoting protein synthesis and wound healing. It also improves immunity and treatment of metabolic disorders in obesity, diabetes, cancer, cardiovascular diseases, ischemia reperfusion injuries and various inflammatory diseases (Wang *et al.*, 2013). The concentration of glycine in the seeds ranged from 3.99±0.02 (unfermented) to 4.40±0.08 g/100g protein (fermented for 24 h). These values showed that this amino acid decreases in concentrations with fermentation period. The glycine values obtained in this work are lower than the 5.92 g/100g protein reported for *Parkia biglobosa* by Oluwaniyi and Bazambo (2016). However, they were higher compared than the 1.21 (unfermented) to 1.69 g/100g protein (fermented for 48 h) range reported for African oil beans by Okechukwu *et al*. (2012). Arginine plays important role in cell division, wound healing, ammonia removal, immune function and hormone release. It is also the precursor for biological synthesis of nitric oxide which plays important roles in neurotransmission, blood clotting and maintenance of blood pressure (Sarma *et al*., 2013). The contents of arginine in the samples in this study ranged from 5.68±0.02 in unfermented to 6.10±0.08 g/100g protein in fermented for 24 h. The increase observed in arginine contents of the samples with the fermentation period, might have been as a result the formation of intermediate compounds during metabolism which reacted with ammonia and were converted to amino acids which were useful in the formation of other amino acids (Okechukwu *et al.*, 2012). The values of arginine in this work were higher than the 2.74±0.05 g/100g protein reported for *A. fasciatus* by Furuya *et al.* (2015). Also, the values were higher compared to the arginine concentration of groundnut: 2.89±0.06 (unfermented) to 3.04±0.05 g/100g protein (fermented for 30 h) reported by Bujang and Taib (2014).

However, the values were lower when compared to the arginine content analyzed for *P. africana* seeds flour: 41.60±1.00 (unfermented) to 49.00±0.51 g/100g protein (fermented for 72 h) reported by Igwe *et al.* (2012). The proline contents of the unfermented seeds ranged from 3.65±0.09 (unfermented) to 4.20±0.02 g/100g protein (fermented for 24 h). These values were similar compared to 4.09 (unfermented) to the 4.28 g/100g protein (fermented for 48 h) reported for African locust beans flour by Ijarotimi and Keshinro (2012) while the values were higher compared to the 1.50±0.30 g/100g protein reported for *Stolephorus commersonii* by Mohanty *et al*. (2015). Although, the values were lower when compared with the 38.00±0.31 g/100g protein reported for *P. africana* by Igwe *et al*. (2012). The cysteine contents ranged from 1.09±0.06 (unfermented) to 2.00±0.05 g/100g protein (fermented for 24 h).

It can be inferred that the samples had lower values than the 21.20±0.21 g/100g protein reported for *P. africana* by Igwe *et al*. (2012). However, the values were higher compared with the 0.40 g/100g protein (unfermented) to 1.40 g/100g protein (fermentation at 48 h) reported for African oil beans by Okechukwu *et al*. (2012). The increased observed in cysteine during the fermentation period might have been as a result of formation of intermediate compounds during metabolism that reacted with ammonia and were converted to amino acids which in turn were useful in the formation of other amino acids (Okechukwu *et al*., 2012). Tyrosine concentration ranged from 3.53±0.03 (unfermented) to 3.94±0.02 g/100g protein (fermented for 24 h). These values were higher compared with 1.18±0.01 g/100g protein reported for *A. faciatus* by Furuya *et al*. (2015). Also, the values are higher compared with the 0.20±0.00 g/100g protein for *Stolephorus commersonii* reported by Mohanty *et al*. (2014). Although, the values were lower compared with the fermented value of African oil bean reported by Okechukwu *et al.* (2012). In addition, the values were high than 33.40±0.30 g/100g protein (unfermented) to 40.60±0.51 g/100g protein (fermentation at 48 hours) reported for *P. africana* by Igwe *et al.* (2012). The concentration of total conditionally essential amino acids content of 21.47±0.10 (fermented for 24 h) to 22.38±0.10 g/100g protein (unfermented). All these values were much higher than the 16.11 g/100g protein (unfermented) to 17.89 g/100g protein (fermented for 48 h) reported for African locust beans flour by Ijarotimi and Keshinro (2012). The serine concentration was 3.83±0.06 (unfermented) to 4.25±0.02 g/100g protein (fermented for 48 h) were recorded for the samples. Base on the result of the serine contents analyzed in this work, it was recorded that the concentration of this acid decrease on fermentation for 24 h while when fermentation exceeded that period the concentration serine decreased. These results were higher than the respective values of the 1.22±0.01 and 1.98±0.01 g/100g protein (for unfermented and fermented for 30 h) reported for groundnut seed by Bujang and Taib (2014). These values were lower than 46.80±0.60 g/100g protein (unfermented) to 64.40±1.01 g/100g protein (fermented for 72 h) reported for *Prosopis africana* by Igwe *et al*. (2012). Since these values are high in those samples, they can serve as sources of this amino acid. Glutamic acid plays an important role in amino acid metabolism because of its role in transamination reactions and is necessary for the synthesis of key molecules, such as polyglutamate folate cofactor and glutathione which are required for the removal of highly toxic peroxides (Wu, 2013). The glutamic acid values of 11.96±0.08 (unfermented) to 13.53±0.08 g/100g protein (fermented for 24 h). It was observed that, glutamic acid concentrations had the highest contents among the analyzed amino acids in this work. The values obtained here are lower than the 102.20±4.10 g/100g protein reported for *Prosopis africana* by Igwe *et al*. (2012). Also, the values are lower when compared with the 16.90 g/100g protein recorded for African oil beans by Okechukwu *et al*. (2012) but these values are higher than the 4.85±0.15

g/100g protein (unfermented) to 5.77±0.32 g/100g protein (fermented for 30 h) reported for garbanzo bean by Bujang and Taib (2014). However, African locust beans flour had similar values of 14.82±0.01 g/100g protein as reported by Ijarotimi and Keshinro (2012).

The aspartic acid concentration ranged from 9.40±0.05 (unfermented) to 9.86±0.04 g/100g protein (fermented for 24 h). Similar values were reported for African oil beans (10.12 g/100g protein) by Okechukwu *et al*. (2012). The values obtained in this work were lower than the 22.82±0.01 g/100g protein (unfermented) to 23.15±0.02 g/100g protein reported for African locust bean flour by Ijarotimi and Keshinro (2012). These values were however, high when compared with the 3.64±0.01 g/100g protein reported for *A. fasciatus* by Furuya *et al*. (2015). Alanine contents ranged from 4.70±0.06 (unfermented) to 5.32±0.09 g/100g protein. Fermentation for 48 h significantly decreased in the amino acids contents. The value obtained in this work was low compared with the 7.80±1.10 g/100g protein reported for *Labeo rohita* by Mohanty *et al*. (2014). The total non-essential amino acids ranged from 34.33±0.09 (fermented for 24 h) to 37.29±0.11 (unfermented) %, respectively. These values were lower compared to the 43.12% reported for fermented *P. africana* seeds by Igwe *et al.* (2012).

Table 4: Anti-nutrient compositions (mg/100g) of African custard apple

Parameters	Unfermented	Fermented for 24 h	Fermented for 48 h
Phytate	18.02±0.40[c]	15.45±0.34[b]	11.55±0.22[a]
Oxalate	13.00±0.50[c]	10.32±0.26[b]	7.56±0.16[a]
Cyanide	9.12±0.33[c]	7.18±0.22[b]	4.00±0.20[a]

Mean within a row with same superscripts were not significantly different at ($p \geq 0.05$)

The levels of the anti-nutrient contents of fermented and unfermented seed flour as presented in Table 4. The fermentation at longer periods reduced the level of the anti-nutritional composition of the plant seeds. The phytate contents ranged from 11.55±0.22 (fermented for 48 h) to 18.02±0.40 mg/100g for unfermented. These were higher than the 0.04 mg/100g (fermentation for 4 days) and 0.01 mg/100g (fermented for 14 days) reported for African oil beans seed by Balogun (2013). The values were however, low compared to 41.77±0.31 mg/100g (unfermented) to 16.94±0.01 mg/100g (fermentation at 24 h) reported by Abdulrahman *et al*. (2016). Base on the phytate concentration, the plant seeds both fermented and unfermented could be consumed without much fear of phytic acid toxicity.

The lethal dose of oxalates is between 200 and 500 mg/100g (NRC, 2013). The oxalate contents ranged from 7.56±0.16 (fermented for 48 h) to 13.00±0.50 mg/100g (unfermented). The values were higher than the 1.05±0.07 mg/100g (unfermented) to 1.02±0.21 mg/100g (fermentation at 72 hours) reported for soybeans by Babalola and Giwa (2012) but lower than the value for African bean seed (180.00±1.15 mg/100g) reported by Abdulrahman *et al*. (2016). The oxalate contents of both fermented and unfermented plant seed flour suggested that, they could be safe for consumption since they all fell below the lethal dose limit. The cyanide contents ranged from 4.00±0.20 (fermented for 48 h) to 9.12±0.33 mg/100g (unfermented). These values were lower than the 19.23±0.13 mg/100g reported for lima bean seeds by Adegbehingbe *et al*. (2014). However, these values were higher than the 0.032±0.007 mg/100g reported for *Citrullus vulgaris* by Peter-Ikechukwu *et al.* (2015).

Conclusion

The results of this study have generally revealed that fermentation, especially after 48 h improved most of the nutritional qualities of the test samples. Also, the anti-nutrient factors of the samples were reduced thereby making them more useful in nutritional applications. The study has also revealed that *A. senegalensis* seeds if properly processed can be useful for food supplementation.

References

Abdulrahman BO, Osibemhe M & Idoko AS 2016. The status of mineral and anti-nutritional composition of raw and fermented seeds of African locust bean (*Parkia biglobosa*), *Int. J. Cur. Res. Bios. and Plant Bio.*, 3(2): 1 – 4.

Adamu GOL, Ezeokoli OT, Dawodu AO, Adebayo-Oyetoro AO & Ofodile LN 2015. Macronutrients and Micronutrients Profile of Some Underutilized Beans in South Western Nigeria, *Int. J. Biochem. Res. & Review*, 7(2): 80-89.

Adebowale OJ & Maliki K 2011. Effect of fermentation period on the chemical composition and functional properties of Pigeon pea *(Cajanus cajan)* seed flour. *Int. Food Res. J.*, 18(4): 1329-1333.

Adegbehingbe KT, Fatusa B, Adetuyi FC & Akinyosoye FA 2014. Effect of fermentation on nutrient and anti-nutrient contents of ground-cooked lima bean (*Phaseolus lunatus*) seeds using *Bacillus subtilis* and *Bacillus pumilus*. *Brit. Micro. Res. J.*, 4(11): 1285-1298.

Alqasim AM 2013. *Annona senegalensis* persoon: A Multipurpose shrub, its phytotherapic, phytopharmacological and phytomedicinal uses. *Int. J. Sci. and Tech.*, 2(12): 862-865.

AOAC 2006. Official Methods of Analysis of AOAC Instrumental, 18th ed., Current through

Revision 1. AOAC International. Gaithersburg, MD, USA, pp. 1-70.

Aremu MO, Awala EY, Opaluwa OD, Odoh R & Bamidele TO 2015. Effect of processing onnutritional composition of African locust bean (*Parkia biglobosa*) and mesquite bean (*Prosopis africana*) seeds, *Comm. in Appl. Sci.*, 3(1): 22-41.

Audu SS & Aremu MO 2011. Effect of processing on chemical composition of red kidney beans (*Phaseolus vulgaris* L.) flour. *Pak. J. Nutr.*, 10(11): 1069-1075.

Babalola RO & Giwa OE 2012. Effect of fermentation on nutritional and anti-nutritional properties of fermenting Soy beans and the antagonistic effect of the fermenting organism on selected pathogens. *Int. Res. J. Micro.*, 3(10): 333-338.

Balogun BI 2013. Evaluation of the nutritional potentials of fermented oil beans seed *Pentaclethra macrophyllah* Benth. *Pharm. Nsuka J.*, 9(2): 73 – 87.

Bello MO 2014. *Nutrient potentials of underutilised plant species in Nigeria*. United Nations University Institute for Natural Resources in Africa, Accra, Ghana, pp. 1 – 52.

Burkill HM 2000. *The Useful Plants of West Tropical Africa*, Vol. 5. Royal Botanic Gardens,

Kew, 296-297.

Bujang A & Taib NA 2014. Changes on amino acids content in soybean, garbanzo bean and groundnut during pre-treatments and *Tempe* making. *Sains Mala.*, 43(4): 551–557.

Coates PK 2002. *Trees of southern African*. Struik Publishers, Cape Town.

Costa J, Gomes C & Carvalho M 2010. Diagnosis, pathogenesis and therapeutic targets in amyotrophic lateral sclerosis. *Neur. Disor. Drug Tar.*, 9: 764-778.

Ebert AW 2014. Potential of underutilized traditional vegetables and legume crops to contribute to food and nutritional security, income and more sustainable production systems. *Sustain.,* 6: 319-335.

FMH 2016. Federal Ministry of Health *saving newborn lives in Nigeria: Newborn health in the context of the integrated maternal, newborn and child health strategy,* Second edition, 2016.

Franzo J, Hunter D, Borelli T & Mattei F 2013. *Diversifying food and diets using agricultural biodiversity to improve nutrition and health*. Routledge, London and New York, p. 401.

Furuya WM, Michelato M, Salaro AL, da Cruz1 TP & Barriviera-Furuya VR 2015. Estimation of the dietary essential amino acid requirements of colliroja *Astyanax fasciatus* by using the ideal protein concept. *Lat. Am. J. of Aq. Res.,* 43(5): 888-894.

Harbome JB 1998. Phytochemical Methods. In: A Guide to Modern Techniques of Plant Analysis 3rd ed., pp. 40-137.

Igwe CU, Ojiako OA, Anugweje KC, Nwaogu LA & Ujowundu CO 2012. Amino acid profile of raw and locally processed seeds of *Prosopis africana* and *Ricinus communis*: Potential antidotes to protein malnutrition. *Fun. Foods in Health and Dis.,* 2(4): 107-119.

Ijarotimi OS & Keshinro OO 2012. Comparison between the amino acid, fatty acid, mineral and nutritional quality of raw, germinated and fermented African locust bean (*Parkia biglobosa*) flour. *Sci. Pol. Tech. Al*., 11(2): 151-165.

Jolaoso AA, Ajayi JO, Ogunmuyiwa SIO & Albert OM 2014. Functional, proximate and mineral composition of a lesser known fermented legume (fermented seeds of *Cathormion altissimum*, Oso), *J. of Emer. Tr. in Engin. and Appl. Sci.,* 5(2): 129-134.

Liao SM, Du QS, Meng JZ, Pang ZW & Huang RB 2013. The multiple roles of histidine in protein interactions. *Chem. Cen. J.,* 7: 44-55.

Mathew TJ, Ndamitso MM, Otori AA, Shaba EY, Inobeme A & Adamu A 2014. proximate and mineral compositions of seeds of some conventional and non-conventional fruits in Niger State, Nigeria. *Academic Res. Int.,* 5(2): 113-118.

Mbaeyi-Nwaoha IE & Obetta FC 2016. Production and evaluation of nutrient-dense complementary food from millet (*Pennisetum glaucum*), pigeon pea (*Cajanus cajan*) and seedless breadfruit (*Artocarpus altillis*) leaf powder blends. *Afr. J. Food Sci.,* 10(9): 143 -156.

Mohanty B, Mahanty A, Ganguly S, Sankar TV, Chakraborty K, Rangasamy A & Sharma AP 2014. Amino acid compositions of 27 food fishes and their importance in clinical nutrition. *J. of Am. Ac.*, 7: 1 – 8.

Monirujjaman M & Ferdouse A 2014. Metabolic and physiological roles of branched-chain amino acids. *Adv. in Mol. Bio.,* 5(3): 6-11.

Mosisa MT 2017. Effect of processing on proximate and mineral composition of black climbing (*P. coccineus* L.) bean flour, *Afr. J. Food Sci.*, 11(3): 74 – 81.

National Research Council (NRC) 2013. Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic: Interim Report. National Academy of Sciences, Washington, 117 pp.

Nieman DCD, Butterwortm E & NiemanCN 1992. Nutrition. Wm. C. Brown Publishers, Dubuque, IA., USA, p. 540.

Ogunyinka BI, Babatunji E, Oyinloye BE, Osunsanmi FO, Kappo AP & Opoku AR 2017. Comparative study on proximate, functional, mineral, and antinutrient composition of fermented, defatted, and protein isolate of *Parkia biglobosa* seed. *Food Sci. & Nutr.* 5(1): 139–147.

Okechukwu RI, Ewelike NC, Ukaoma AA, Emejulu AA, & Azuwike CO 2012. Changes in the nutrient composition of the African oil bean meal "ugba" (*Pentaclethre Macrophylla* Benth) subjected to solid state natural fermentation. *J. of Appl. Bios.* 51: 3591–3595.

Oladele EP & Oshodi AA 2008. Effect of fermentation on some chemical and nutritive properties of berlandier nettle spurge (*Jatropha cathartica*) and physic nut (*Jatropha curcas*) seeds. *Pak. J. Nutr.* 7(2): 292 – 296.

Oluwaniyi OO & Bazambo IO 2016. Nutritional and amino acid analysis of raw, partially fermented and completely fermented locust bean (*Parkia biglobosa*) seeds. *Afr. J. of Food Agri., Nutr. & Devt.* 16(2): 10866 – 10883.

Omodara TR & Olwomofe TO 2015. Effect of fermentation on the nutritional quality of African locust bean and soya bean. *Int. J. Sci. Res.,* 2319-7064.

Peter-Ikechukwu AI, Enwereuzoh RO, Uzoukwu AE, Nze SM, Agunwa MI, Anagwu FI & Onyemachi C 2015. Effect of fermentation on the anti-nutritional factors and mineral composition of melon seed varieties for *Ogiri* production. *Food Sci. & Qual. Mgt.,* 43: 98 – 111.

Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N & Dougherty DM 2009. L-tryptophan: Basic metabolic functions, behavioral research and therapeutic indications. *Int. J. Trypt. Res.,* 2(1): 45–60.

Ripps H & Shen W 2012. Review: Taurine: A very essential amino acid. *Molecular Vision*, 18: 2673-2686.

Sarma D, Akhtar MS & Das P 2013. Nutritional quality in terms of amino acid and fatty acid of five coldwater fish species: Implications to human health. *Nat. Acad. Sci. Lett.,* 36(4): 385–391.

Thingom P & Chhetry GKN 2011. Nutritional analysis of fermented soybean (*Hawaijar*). *Assam Uni. J. Sci. & Tech.*, 7(1): 96-100.

Wang W, Wu Z, Dai Z, Yang Y, Wang J & Wu G 2013. Glycine metabolism in animals and humans: Implications for nutrition and health, *Am. Ac.*, 45(3): 463–477.

Wu G 2010. Functional amino acids in growth, reproduction, and health. *Adv. in Nutr.*, 1(1): 31 – 37.

Wu G 2013. Functional amino acids in nutrition and health. *Am. Ac.*, 45(3): 407–411.