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Protein Levels and Amino Acids Composition in Some Leaf Vegetables Sold at Wukari in Taraba State, Nigeria

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Abstract

This study was conducted to determine the protein and energy levels, and also amino acids content in some leafy vegetables. Amino acids composition was determined using High Performance Liquid Chromatography (HPLC) method, energy determination was by the bomb calorimetric method while other analyses carried out on the vegetables were adapted from well-known standardized methodologies by Association of Official Analytical Chemists (AOAC). The result obtained shows that moisture content in the leafy vegetables ranged between 10.20±0.00 and 10.875 ± 0.0071% with water leaf (*Talinum triangulare*) having the highest mean percentage water content of 10.875±0.0071%. Also, protein values in the leafy vegetables gave a range of 11.1726±0.0232 to 25.036±0.0078% with the highest mean protein content of 25.036±0.0078% obtained in pumpkin leaf (*Telfairia occidentalis*). The amino acid composition showed the following range in individual leaf vegetables: 1.035 ±0.0071 - 5.415±0.0071 g/100 g in spinach Leaf (*Amaranthus hybridus)*, 1.14±0.0071 - 7.83±0.0141 g/100 g in bitter leaf (*Vernonia amygdalina*), 1.26±0.0141 - 8.745±0.0071 g/100 g in pumpkin leaf (*T. occidentalis)* and 0.985±0.0071-4.96±0.014 g/100 g in water leaf (*T. triangulare).* It was discovered that the energy content was highest in *T. occidentalis* (216.365±0.0212 Kcal/100g while the least was recorded for water leaf (153.745±0.0071 Kcal/100g). Based on the findings in the analysis carried out in this study, it can be concluded that *T. occidentalis* appeared to have superior nutritional values than other leaf vegetables analyzed.

1. Introduction

Green foliage vegetables, especially the known leafy ones consumed in Nigerian household are indispensable part of many culinary preparations in various household. Many of these leaf vegetables are found all over the country, however, there are particular types easily associated by some regions due to the importance attached to them and rate of consumption in such regions in Nigeria. It is well known that vegetables abound in nutrients especially minerals and vitamins which serve as sources of replenishment of these nutrients in humans who need them on daily basis for proper metabolic and biochemical functions. According to Omoyeni *et al.* [1] studies have repeatedly shown that increasing colon and stomach cancer correlate with low vegetable meals and it has been suggested that vegetables may help resist them.

There is also increasing epidemiological evidence in favour of an association between nutrition and susceptibility to infection. Health disorders such as appendicitis, hemorrhoids, gall stones, heart diseases, obesity and constipation can be either corrected, or treated by copious consumption of vegetables [2]. According to Aregheore, [3] green leafy vegetables provide cheap and abundant sources of proteins. Vegetables can also synthesize amino acids from a wide range of available primary materials such as carbon dioxide, water and atmospheric nitrogen as in legumes [4-11]. *Vernonia amygdalina* used as a fence post and pot-herb in the home and villages is one of the most widely consumed leaf vegetables in most countries in West and Central Africa [12]. *V. amygdalina* plant commonly found around homes in Southern Nigeria as a green vegetable or spice especially in the popular "bitter-leaf soup" [13]. *Amaranthus hybridus* is known to people from South Eastern Nigeria as 'inene' and it belongs to the family Amaranthacae [14]. Spinach (*A. hybridus*) tolerates varying soil and climatic conditions but altitudes of over 1500 ft are unsuitable [15]. Fluted pumpkin (*Talfairia occidentalis* Hoof) is a tropical vine grown primarily for the leaves and edible seed as an important component of food of many people in West Africa [16]. Housewives in Nigeria prefer the female leaves of *T. occidentalis* leading to higher demand [17]. The young shoots and leaves of the female plant form main ingredients in *edikangikong*, a soup favoured by people in Cross River and Akwa Ibom state, Nigeria [17]. Fluted pumpkin commonly known as 'ugu' is the most sought after vegetable used in all culinary activity in Eastern Nigeria. Water leaf (*Talinum triangulare*) is a common vegetable cultivated and consumed in all parts of Nigeria in combination with other vegetables such as bitter leaf and fluted pumpkin in vegetable soup where it is believed to provide much needed softness in texture of such soups considering that other vegetables are relatively hard. It is also prepared with tomatoes when used in stew preparation. This research is aimed at quantification of the level of proteins and amino acid composition in some selected leafy vegetables popularly sold in Wukari market, Taraba State, Nigeria.

2. Materials and Method

2.1. Study Location

The study was conducted at Federal University Wukari in Taraba State, which is located in north-eastern region of Nigeria on Longitude 9.783°E and Latitude 7.850°N. The physico-chemical analysis was carried out at the Department of Animal Production Laboratory of Adamawa State University, Mubi, Adamawa State.

2.2. Amino Acid Assay

Amino acid analysis was carried out with the aid of High Performance Liquid Chromatography (HPLC) equipped with UV 338 nm detector, column with C18, 2.5 x 200 mm, 5 µm column and a mobile phase of 1:2:2 (100 mM sodium sulphate, pH 7.2; acetonitrile; methanol (v/v/v) at a flow rate of 0.45 ml/min and an operating temperature of 40°C.

2.3. Sources of Leaf Vegetable Samples

The leafy vegetables used for this project were purchased from new market at Wukari in Taraba State. These leaf vegetables are; bitter leaf (*Vernonia amygdalina*), spinach leaf (*Amaranthus hybridus*), pumpkin leaf (*Telfiaria occidentalis*), and water leaf (*Talinum triangulare*).

2.4. Treatment of Sample

The harvested individual leaf vegetable samples were washed using clean distilled water, destalked, mixed together and air-dried. The dried samples of each leaf vegetable were mixed and kept in a polythene bag in a cool and dried place pending analysis.

2.5. Physico-Chemical Analysis

Standard methodologies by Association of Official Analytical Chemists [18] were used to determine:

2.6. Protein Analysis

Protein levels in samples were carried out using the Kjeldahl nitrogen determination of protein with results adjusted through calculation as:

$$\%\, N\ = \frac{0.00014\ \times Titre\ value\ \times 50\ \times 100}{Wt.of\ sample\ taken}$$

$$\%\, Protein\ = N\ \times\ 6.25$$

2.7. Moisture Content Determination

Moisture contents of the various leaf vegetable were determined by using oven (GenlabMiNO/30 UK). Differences in weight are calculated as:

$$\%\, Moisture = \frac{(Wt\ of\ dish + Sample\ before\ Drying) - (Wt\ of\ Dish + Sample\ after\ Drying) \times 100}{Wt\ of\ Sample\ Taken}$$

2.8. Gross Energy Composition Determination

The gross energy composition of the samples was determined using Gallen Kamp oxygen ballistic bomb calorimeter. Calculate the energy value of the sample in kcal per gram of sample [18].

3. Result

All the leafy vegetables showed appreciable energy content (Table 1) with highest mean energy ($216.37\pm0.02\ kcal/100\ g$) obtained in pumpkin leaves. The mean water content per dry mass in all the leaves was below 11% in all samples (Table 2). The highest (25.04±0.01%) and lowest (11.17±0.02% mean protein percentage were obtained in pumpkin and water leaves respectively (Table 3). Amino acid analysis (Table 4) shows mean amount of its contents. The amino acid composition showed the following range in individual leaf vegetables: 1.035 ±0.0071 - 5.415±0.0071 g/100 g in spinach leaf (*A. hybridus)*, 1.14 ± 0.0071 - 7.83 ± 0.0141 g/100 g in bitter leaf (*V. amygdalina)*, 1.26 ± 0.0141 - 8.745 ± 0.0071 g/100 g in pumpkin leaf (*T. occidentalis)* and $0.985\pm0.0071 - 4.96\ \pm 0.014$ g/100 g in water leaf (*T. triangulare).*

Table 1. *Energy composition of samples analyzed.*

Samples	Mean (kcal/100 g))
Pumpkin leaves	216.37±0.02
Spinach	187.55±0.01
Water leaves	153.75±0.01
Bitter leaf	197.87±0.02

Table 2. *Moisture content of sample analyzed.*

Sample	Mean (%)
Pumpkin leaves	10.75±0.00
Spinach	10.53±0.01
Water leaf	10.88±0.01
Bitter leaf	10.20±0.00

Table 3. *Protein composition of samples analyzed on dry matter basis.*

Samples	Mean (%)
Pumpkin leaves	25.04±0.01
Spinach	16.28±0.02
Water leaf	11.17±0.02
Bitter leaf	18.41±0.02

Table 4. *Amino Acid Profile.*

Amino acid	Mean (g/100 g) Pumpkin (*T. occidentalis*)	Mean (g/100 g) Spinach leaf (*A. hybridus*)	Mean (g/100 g) Bitter leaf (*V. amygydalina*)	Mean (g/100 g) Water leaf (*T. triangulare*)
Lysine	3.71±0.01	2.12±0.01	3.42±0.01	2.84±0.01
Threonine	2.11±0.01	1.44±0.03	2.06±0.01	1.24±0.01
Cysteine	1.26±0.01	1.04±0.01	1.15±0.01	0.99±0.01
Valine	4.12±0.02	2.65±0.01	3.99±0.01	2.17±0.01
Tryptophan	5.35±0.01	3.88±0.01	4.67±0.01	3.13±0.02
Methionine	2.26±0.01	1.455±0.01	1.98±0.00	1.29±0.02
Isoleucine	2.53±0.01	1.77±0.01	2.15±0.01	1.86±0.01
Leucine	5.95±0.01	3.57±0.01	5.11±0.01	2.97±0.01
Tyrosine	2.87±0.01	1.56±0.01	2.58±0.01	2.21±0.01
Phenylalanine	3.52±0.01	2.96±0.01	3.65±0.01	3.42±0.01
Histidine	2.28±0.01	1.34±0.01	2.38±0.01	1.07±0.01
Arginine	3.97±0.01	3.01±0.01	3.84±0.01	2.88±0.01
Aspartic acid	6.83±0.01	5.42±0.01	6.55±0.01	4.96 ±0.01
Serine	4.32±0.01	2.77±0.01	4.55±0.01	1.87±0.01
Glutamic acid	8.75±0.01	5.05±0.01	7.83±0.01	4.75±0.00
Proline	2.13±0.02	1.27±0.00	3.63±0.01	1.26±0.01
Glycine	2.87±0.01	1.39±0.01	2.76±0.01	1.35±0.01
Alanine	2.76±0.01	1.09±0.01	2.52±0.02	2.27±0.01

4. Discussion

4.1. Moisture Content

Water is one of the major nutrients required by the body for proper development. It aids in digestion and absorption of food. Water constitutes by far bulk of each fresh vegetable or leguminous substance for absorption by plants, as food is dependent on the presence of water [19]. According to Fontana, [20] the water activity (moisture content) of a food describes the energy state of water in the food, and hence its potential to act as a solvent and participate in chemical/biochemical reactions and growth of microorganisms. It is an important property that is used to predict the stability and safety of food with respect to microbial growth, rates of deteriorative reactions and chemical/physical properties. The moisture content of food item could be used as an index of stability and susceptibility to fungal and bacterial infection [21]. Result obtained for moisture determination of pumpkin, spinach, water, and bitter leaves studied showed that the level of moisture content in each of the leaves by dry mass estimation are similar and moderately high showing values greater than 10% and below 11 (Table 2). In the analysis carried out, it was discovered that water leaf has the highest water content ($10.88\ \pm0.01\%$) compared to other samples. The moisture content values obtained in this work was lower than those of Adegunwa *et al.* [22], whose analysis of bitter leaf (*V. amygdalina*), fluted pumpkin (*T. occidentalis*), water leaf (*T. triangulare*), and indian spinach (*Basella alba*) with dry matter content ranged from 35.5% to 39.3%. The high-water content of leafy vegetables may therefore be responsible for their higher rate of perishability since favourable

environment is provided for fungi and bacteria deterioration. Compared to similar analysis elsewhere as explained above, water content from the leafy vegetable in this study showed that it may have better shelf life. Diet wise, good quantity of water is provided by the leafy vegetables which is sufficient in biochemical reactions during human metabolism.

4.2. Crude Protein

The protein content obtained is known as "crude protein" because most other forms of nitrogen may be digested as protein such as nucleic acids and nitrogen [23]. About 80% of protein from vegetables sources is absorbed by the body. Crude protein levels obtained in this research were higher in pumpkin leaves (*T. occidentalis*) (25.036±0.0078%) than in other samples: spinach (*A. hybridus*) (16.28±0.02%), water leaf (*T. triangulare*) (11.17±0.02%) and bitter leaf (*V. amygdalina*) (18.41±0.02%). This value is corroborated by the findings of Mohammed and Mann, [24] whose crude protein value obtained from *T. occidentalis* (21.95%) was slightly lower than the value obtained in this work. Also, the level of crude protein is much higher in the analyzed pumpkin leaves in this research than in the level (3.15g/100 g) obtained for nutritive value of pumpkin leaves elsewhere [25]. However, the values obtained by Adegunwa *et al.* [22], showed that the protein content obtained in sun-dried vegetables samples ranged from 23.4% in *V. amygdalina* to 31.1% in *T. accidentalis*. In their blanched and sun-dried samples, the protein content ranged from 19.5% in *T. triangulare* to 23.4% in *T. occidentalis*. Other values of crude protein in leafy vegetables analyzed obtained elsewhere [23] were higher than those of this research with the exception of pumpkin leaves (*T. occidentalis*). Variation in soil nutrient, environmental influence, and period of cultivation could be the reason for the differences observed. Generally, the activities of enzymes involved in metabolic pathways like the nitrate reductase enzyme, carboxypeptidases, amino peptidases and endopeptidases are higher in the pumpkin leave [26]. In adults, more than 250 g of protein are synthesized and degraded per day, compared to an average daily intake of approximately 55 - 100 g per day [27]. This therefore means that there is need for steady replacement of protein and leaf vegetables could be ideal source of replenishment of extra protein thus needed. According to the Food and Nutrition Board (FNB) at the Institute of Medicine, the recommended dietary allowance of protein for both adult men and women is 800 mg of good quality protein per kg of body weight per day [28]. Implication of the finding is that all the vegetables examined have moderate contents of protein while the pumpkin leaves indisputably source of choice for protein content in vegetables

4.3. Calories

In the analysis carried out, it was observed that fluted pumpkin has the highest level of calories compared to other samples (Spinach (187.55±0.01 kcal/100 g), Water leaf (153.75±0.01 kcal/100 g) and bitter leaves (197.87±0.01 kcal/100 g). The fluted pumpkin has about 216.37±0.02 kcal/100 g in terms of energy composition. It is quite possible because the leaves are photosynthetically more active (due to higher chlorophyll content and exposure to sunlight). The energy values of the vegetables obtained by Mohammed and Mann, [24] showed that it was highest in the stem of African spinach (183.93 kcal/100 g) which is comparable but lower than the value obtained in this research. Their lowest gross energy was in the root of water leaf plant (37.73 kcal/100 g) which is lower than the least value of 153.75±0.01 kcal/100 g obtained in the water leaves analyzed in this work. Thus, the energy value agrees with the general observation that vegetables have low energy values [29].

4.4. Amino Acid

Amino acid analysis (Tables 4) show mean amount range of sample contents: pumpkin leaves between 2.11±0.01 to 5.95±0.01% for the essential amino acids and 1.26 ± 0.01 to 8.75 ± 0.01% for the non-essential amino acids (Table 4), spinach leaves between 1.34±0.01 to 3.88±0.01% for essential amino acids and 1.04±0.01 to 5.42±0.01% for non-essential amino acids (Table 4), bitter leaves has range of 1.98±0.00 to 5.11±0.01% for essential amino acids and 1.15±0.01 to 7.83±0.01% for non-essential amino acids, while water leaves has range of 1.07±0.01 to 3.42±0.01% for the essential amino acids and mean range of 0.99±0.01 to 4.96±0.01% for non-essential amino acids (Table 4), respectively.

Although the free amino acids dissolved in plasma and tissue (some gotten from leaf vegetables) represents only a very small proportion of the body's total mass of amino acids, they are critical for the nutritional and metabolic control of the body's proteins. Unlike total body protein, the concentrations of individual free amino acids in body fluids can change substantially in response to dietary variations or pathological conditions [30] thus the leafy vegetables examined in this research could provide specific amino acids needed by the body. The body's capacity to conserve individual amino acids at low intakes varies widely, so the pattern of amino acids needed in the diet to match their individual catabolic rates does not correspond precisely with the composition of body protein [31]. The outstanding essential amino acid in this research was leucine which content is dominant in all the leafy vegetables investigated viz: pumpkin leaf (5.95±0.01%), spinach (3.57±0.01%), bitter leaf (5.11±0.01%), and water leaf (2.97±0.01%). The values derived from consumption of these vegetable is attributable to the leucine content and their importance as explained by Gold, [32] some of which are: healing and repair of muscle tissues, clotting at site of injuries, production of growth hormones, regulation of blood sugar, increasing endurance and provision of energy in the body. Other essential amino acids with appreciable contents obtained in the analyzed vegetables include: tryptophan in pumpkin leaf (5.35±0.01%), spinach (3.88±0.01%), bitter leaf (4.67±0.01%) and water leaf (3.13±0.02%) and

phenylalanine in pumpkin leaf (3.51±0.01%), spinach (2.96±0.01%), bitter leaf (3.65±0.01%) and water leaf (3.42±0.01%). Gold, [32] explained that phenylalanine is needed in treating brain disorder, normal functioning of the central nervous system, control of symptoms of depression and chronic pain, while tryptophan is important in the manufacture of neurotransmitter serotonin, which regulates mood and sleep pattern, treatment of jet lag, depression and binge eating. It is also required for treating vascular migraines, panic attacks, rheumatoid arthritis, and normal functioning of the central nervous system. The most notable non-essential amino acids in this research were: aspartic acid in pumpkin leaf (6.83±0.01%), spinach (5.42±0.01%), bitter leaf (6.55±0.01%), and water leaf (4.96±0.01%) and glutamic acid in pumpkin leaf (8.75±0.01%), spinach (5.05±0.01%), bitter leaf (7.83±0.01%) and waterleaf (4.75 ± 0.00%). Cysteine is observed to be the limiting amino acid in this research showing the lowest content of 0.99±0.01% in water leaf. In some circumstances such as illness or non-availability of enzymes, some non-essential amino acids, such as glutamine, tyrosine, and arginine, may not be synthesized by the body. When this occurs, such non-essential amino acids are said to be conditional essential amino acids and thus must be provided through food intake. This is why even the non-essential amino acids from leaf vegetables as understudied are important should such situation arise.

5. Conclusion

This research shows that the leafy vegetables studied contain appreciable levels of calories, protein, and amino acids. They are also good sources of quality protein and amino acids. Considering the levels of protein and amino acids composition in these leaf vegetables, they may be useful as sources of cheap and quick amino acid and protein replenishment for malnourished children especially pumpkin leaves with high protein levels and good quality amino acids, coupled with high energy composition. However, in as much as some leaf vegetables show exceptionally good qualities of amino acid, it is advisable to consume different varieties so as to provide the body with the assorted amino acids required for proper functioning.

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References

[1] Omoyeni, O. A., Olaofe, O and Akinyeye, R. O. (2015). "Amino acid composition of ten commonly eaten indigenous leafy vegetables of South-West Nigeria." *World Journal of Nutrition and Health*, 3 (1): 16-21. doi: 10.12691/jnh-3-1-3.

[2] Iniaghe, O., Malomo, S., Adebayo, J. and Arise R. (2009). Proximate composition and phytochemical constituents of leaves of some *Acalypha* species. *Pakistan Journal of Nutrition* 8 (3): 256-258.

[3] Aregheore, E. A. (2012). Nutritive value and inherent anti-nutritive factors in four indigenous edible leafy vegetables in human nutrition in Nigeria: A review. *Journal of Food Resource Science*, 1 (1): 1-14.

[4] Lewis, J and Fenwick, G. R. (1987). Glucosinolate content of *Brassica* vegetables: Analysis of twenty-four cultivars of calabres. *Food Chemistry,* 25 (4): 259-268.

[5] Aletor, V. A and Adeogun, O. A. (1995). Nutrients and anti-nutrient components of some tropical leafy vegetables. *Food Chemistry*, 54 (4): 375-379.

[6] Ladeji, O., Okoye Z. S. C. and Ojobe T. (1995). Chemical evaluation of the nutritive value of leave of fluted pumpkin (*Telfairia occidentalis*). *Food. Chemistry*, 53 (4): 353-355.

[7] Fasuyi, A. O (2006). Nutritional potentials of some tropical vegetable leaf meals: Chemical characterization and functional properties. *African Journal of Biotechnology*, 5 (1): 49-53.

[8] Babu, S. C. (2000). Rural nutrition interventions with indigenous plants: A case study of vitamin A deficiency in Malawi. (BASE): *Biotechnologie Agronomie Society Environnement (Belgium)*, 4 (3): 169-179.

[9] Aregheore, E. M. (2002). Intake and digestibility of *Moringa oleifera-batiki* grass mixtures by growing goats. *Small Ruminant Resources*, 46 (1): 23-28.

[10] Ajibade, S. R., Balogun, M. O., Afolabi, O. O and Kupolati, M. D. (2006). Sex differences in the biochemical contents of *Telfairia occidentalis* Hook F. *Journal of Food Agriculture and Environment*, 4 (1): 155-156.

[11] Oduro I, Ellis WO and Owusu D. (2008). Nutritional potential of two leafy vegetables: *Moringa oleifera* and *Ipomoea batatas* leaves. *Scientific Research and Essay*, 3 (2): 57-60.

[12] Ejoh A. R., Tanya A. N., Djuikwo N. A. and Mbofung C. M. (2005). Effect of processing and preservation methods on vitamin C and total carotenoid levels of some *Vernonia* (bitter leaf) species. *African Journal of Food, Agriculture, Nutrition and Development*, 5 (2): 105-117.

[13] Igile, G. O., Olesezk, W., Burda, S. and Jurzysta, M. (1995). Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. Journal Agriculture and Food Chemistry, 43 (8): 2162-2166.

[14] Iheanacho K. M. E. and Udebuani A. C. (2009). Nutrition composition of some leafy vegetables consumed in Imo State, Nigeria. *Journal Applied Science Environmental Management* 13 (3): 35-38.

[15] Tindall, H. D. (1983). Vegetables in the Tropics. 5th edition. Macmillian Publishers London 1452.

[16] Fagbemi, T. N. F., Eleyinmi, A. F., Atum, H. N. and Akpambang, O. (2005). Nutritional composition of fermented fluted pumpkin (*Telfairia occidentalis*) seeds for production of ogiri ugu. Proceedings of the IFT Annual Meeting P 54B-2, Session 54B, Fermented Foods and Beverages: General, July 15-20, 2005, New Orleans, Louisiana.

[17] Aregheore, E. A. (2012). Nutritive value and inherent anti-nutritive factors in four indigenous edible leafy vegetables in human nutrition in Nigeria: A review. *Journal of Food Resource Science*, 1 (1): 1-14.

[18] A. O. A. C. (2000). Association of Official Analytical Chemists. 18th ed, Official Methods of Analysis. Washington D. C: pp. 18-62.

[19] Macmillan, H. F. (1943). Tropical Planting and Gardening with Special Reference to Ceylon. 5th ed. Macmillan, & Co., Ltd., London. Reprinted 1952 pp. 29, 197, 216, 362, 366, 487. (p. 24).

[20] Fontana, A. J. (2001). Water activity's role in food safety and quality. http://www.foodsafetymagazine.com/magazine-archive1/February march-2001/

[21] Scott W. J. (1957). Water relations of food spoilage microorganisms. Advances in Food Research 7: 83-127.

[22] Adegunwa, M. O., Alamu, E. O., Bakare, H. A. and Oyeniyi C. O. (2011). Proximate and bioactive contents of some selected vegetables in Nigeria: Processing and varietal effects. *American Journal of Food & Nutritional*, 1 (4): 171-177.

[23] Suttie, J. W., Carlson J. R. and Faltin. E. C. (1972). Effects of alternating periods of high- and low-fluoride ingestion on dairy cattle. *Journal of Dairy Science*. 55 (6): 790-804.

[24] Mohammed, G. and Mann, A. (2012). Evaluation of the nutritional values of dry season Fadama vegetables in Bida, Nigeria. *African Journal of Food Science*. 6 (11): 302-307.

[25] U. S. D. A. (2016). Full Report (All Nutrients) 11418, Pumpkin leaves, raw National Nutrient Database for Standard Reference. Release 28 slightly revised May, 2016.

[26] Simon, S. and Moss, O. (1987). Nitrate reductase as a factor affecting assimilation during grain filling period in spring wheat. *Crop Sciences*, 18 (4): 584-589.

[27] National Academy of Sciences, (NAS) (2005). Institute of Medicine. Food and Nutrition Board. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids (macronutrients). Available at http://www.nal.usda.gov/fnic/DRI//DRI_Energy/energy_full_report.pdf

[28] Waterlow, J. C. (1984). Protein turnover with special reference to man. *Quarterly Journal of Experimental Physiology*, 69 (3): 409-438.

[29] Lintas C. (1992). Nutritional aspects of fruits and vegetables consumed. *Options Méditerranéennes: Série A. Séminaires Méditerranéens; n.* 19: 29-87.

[30] Furst P. (1989). Amino acid metabolism in uremia. *Journal of American College of Nutrition,* 8 (4): 310-323.

[31] Said, A. K. and Hegsted, D. M. (1970). Response of adult rats to low dietary levels of essential amino acids. *The Journal of Nutrition.* 100 (11): 1362-1375.

[32] Gold C. M. (2009). The Nine essential amino acids. CMG Archives. http://campbelmgold.com.