Comparative Analysis of Minerals, Heavy Metals and Amino Acids Compositions of the Seeds and Juice of *Cucumis metuliferus*

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Authors’ contributions

This work was carried out in collaboration among all authors. Author CEA conceived the work, wrote the protocol and designed the study. Author ONA managed the literature searches and wrote the first draft of the manuscript. Author CKO managed the analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2020/v6i430126

Received 28 April 2020
Accepted 05 July 2020
Published 14 July 2020

ABSTRACT

The aim of this study was to compare the minerals, heavy metals and amino acids compositions of the seeds and juice of *Cucumis metuliferus*. The minerals and heavy metals content were evaluated using FS240AA agilent atomic absorption spectrometer according to the method of American Public Health Association while the amino acids content was evaluated using High Performance Liquid Chromatography (HPLC). From the mineral analysis, concentrations of calcium (23.416 ppm), aluminum (0.094 ppm), manganese (0.242 ppm) and iron (1.243 ppm) were higher in the juice than in the seeds with respective values of 20.084 ppm, 0.079 ppm, 0.221 ppm and 0.934 ppm while the concentrations of magnesium (29.749 ppm), zinc (4.184 ppm), copper (0.125 ppm), sodium (8.927 ppm) and potassium (7.594 ppm) were higher in the seeds than in the juice with respective values of 20.592 ppm, 1.271 ppm, 0.030 ppm, 8.594 ppm and 6.833 ppm. The juice had higher concentrations of heavy metals such as arsenic (20.082 ppm), lead (4.135 ppm), cobalt (0.178 ppm), silver (0.074 ppm), selenium (7.246 ppm) and mercury (4.609 ppm) as against the seed with...
respective values of 0.578 ppm, 1.455 ppm while cobalt, silver, selenium and mercury were not detected. However, the concentrations of cadmium (0.389 ppm), chromium (0.545 ppm) and nickel (0.288 ppm) were higher in the seeds than in the juice with respective values of 0.082 ppm, 0.252 ppm and 0.016 ppm. From the result of amino acid analysis, 18 amino acids were found in both the seeds and juice which include 9 essential and 9 non-essential amino acids respectively. The qualitative composition of amino acids in both the seeds and the juice was same, but the quantitative contents differed although non-significantly from each other with prevalence of amino acids in the seeds. Aspartate was the most abundant of the amino acids found while cysteine was the least. These results suggest that the seeds and juice of Cucumis metuliferus contain adequate essential minerals which are beneficial to human health. The contaminant levels of heavy metals highlights the necessity on the quality and safety concerns about their use and handling. The amino acids analysis showed that both the seeds and juice of Cucumis metuliferus are good sources of amino acid and could be used as food supplement. The amino acid content may also provide useful information for determination of the protein quality of Cucumis metuliferus.

Keywords: Cucumis metuliferus; mineral; amino acid; essential amino acid; nonessential amino acid; heavy metal; seeds; juice.

1. INTRODUCTION

Cucumis metuliferus also called kiwano or horned melon is a specie of the genus; Cucumis. Cucumis is a genus of vines in the gourd family, Cucurbitaceae [1]. It has more than 32 species, indigenous mainly to Africa and also found in Asia, Australia and some islands in the Pacific. The specie name metuliferus refers to the sharp spines on the fruit, from the Latin word, metula, meaning a small pyramid, and ferus, meaning bearing [2]. Cucumis metuliferus grows at an altitude of 210 m to as high as 1800 m above sea level. It flowers from January to May and fruits from February to July. Birds eat the juicy ripe fruits. The fruit is bright orange when ripe with a bright green, gelatinous flesh. Its taste has been compared to a combination of cucumber and banana. It is often eaten raw as a snack, but may also be used in cooking [3,4]. The fruits are peeled and eaten in either the immature or the mature stages. Fruits in the unripe stages have the appearance and taste of cucumber. Mature fruits may have a sweet dessert-fruit flavour. Mature fruits may also be split open and dried in the sun for later use. In Botswana, the Kalahari San people prepare the fruits by roasting [5]. In Western countries, it is mostly marketed as an ornamental for its decorative fruit, with a unique appearance and extended keeping qualities. In some parts of Africa, the leaves are used as a vegetable. In Zimbabwe young leaves are stripped from the stems, washed and boiled as spinach. In Nigeria, the fruits and seeds are eaten raw as supplements [6,7].

The fruits occur in two forms; the bitter and non-bitter forms. Fruits from wild-growing plants are often bitter and inedible and considered poisonous if eaten. The bitter form contains cucurbitacins (triterpenoids), which is a highly toxic compound [8] and are known to exhibit cytotoxic, anti-tumour and anti-inflammatory activities [5]. The bitter compounds of the leaves can be neutralized when they are cooked; the peel is dried or cooked; the seeds are dried and ground into flour to prepare as anti-helminthic against tapeworm [2]. The non-bitter form has been found to be less toxic and has also been widely cultivated [9,10].

The root is used in different parts of Africa for the relief of pain following childbirth [5]. In Benin the fruit is said to possess medico-magical properties and is used to treat eruptive fevers in 'Sakpata voodoo' rituals. The decorticated fruit macerated in distilled palm wine or lemon juice is used to treat smallpox and skin rashes [5]. The sweet varieties of the fruits been used to treat HIV / AIDS positive patients [1]. The fruit is highly sought after as a source of water from people of the Kalahari Desert [11] and is also a good source of energy with low caloric value, being rich in vitamin C and magnesium. In traditional South African medicine all parts of the plant are used: the fresh leaves are chewed, for their energetic and anti-fatigue effect, or they are cooked and eaten, as is generally used for vegetables, or boiled for the preparation of herbal teas with a laxative action, or to prepare anti-inflammatory packs [12].
Studies have shown that the fruit of *Cucumis metuliferus* has blood boosting capacity by producing increase in the production of haemoglobin, PCV and Red blood cell in rats [1]. It has also been reported to increase male fertility [13]; possess *in vitro* antibacterial activity against *Salmonella gallinarum* [14]; antiviral activity [15], antimalarial activity [16] and anti-ulcer action [17]. The glycosides extracted from the pulp possess hypoglycemic activity in alloxan-induced diabetes mellitus in rats [18,19]. From phytochemical analysis of the seed and the rind, it was reported that they contained alkaloids, flavonoids, saponin, steroids, tannin and phenols [20,21]. With the numerous use of *Cucumis metuliferus* fruit, this study was aimed to comparatively assess the minerals, heavy metals and the amino acids content of the seeds and juice which is contained in the pulp of the fruit.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The fruits of *Cucumis metuliferus* were purchased from market in Benue State, Nigeria. The fruits were cut open, the pulp scooped out into a muslin cloth and the juice was squeezed out. The seeds were separated from the pulp and shade-dried at 25°C for two weeks and ground into fine powder using an electric blender. The juice and the powdered mass obtained were subjected to various analyses.

2.2 Analysis of Minerals and Heavy Metals Composition

The minerals and heavy metal analyses were conducted using Varian AA240 Atomic Absorption Spectrophometer according to the method of American Public Health Association (1995).

2.3 Determination of Amino Acid Composition

The determination of the amino acid composition was done using High Performance Liquid Chromatography (HPLC). The sample was hydrolyzed followed by derivatization and then analyzed using HPLC. Thus:

2.3.1 Procedure

2.3.1.1 Hydrolysis

The sample was hydrolyzed by weighing 0.1g lyophilized sample into a 16- × 125-mm screw-cap Pyrex (Barcelona, Spain) tube and adding 15 ml of 6N hydrochloric acid. The tube was thoroughly flushed with Nitrogen, quickly capped, and placed in an oven at 110°C for 24 h. After hydrolysis, the tube contents were vacuum filtered (Whatman #541, Maidstone, England) to remove solids, the filtrate was made up to 25 ml with water, and an aliquot of this solution was further filtered through a 0.50-μm pore-size membrane (Millipore, Madrid, Spain). A standard solution containing 1.25 μmol/ml of each amino acid in 0.1N hydrochloric acid was created.

2.3.1.2 Derivatization

The procedure used was a modification of the method of Elkin et al. [22]. A standard solution (50 μl) was pipetted into a 10- × 5-mm tube and dried in vacuum at 65°C. To the residue, 30 μl of methanol-water-Phenylisothiocianate (2:2:1 [v/v]) was added and then removed in vacuo at 65°C. Next, 30 μl of the derivatizing reagent; methanol-water-Phenylisothiocianate (7:1:1:1 [v/v]) was added, and the tube was agitated and left to stand at room temperature for 20 min. Finally, the solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4°C pending analysis. Prior to injection, 150 μl of diluent consisting of 5 mM sodium phosphate with 5% acetonitrile was added to each tube.

2.3.1.3 Chromatographic procedure

Chromatography was carried out at a constant temperature of 30°C using a gradient elution.

2.4 Statistical Analysis

Data analysis was performed using SPSS version 7 statistical package. Statistical significance of the results between groups was determined using one way analysis of variance (ANOVA). Differences between means were considered significant at P<0.05.

3. RESULTS

3.1 Mineral Composition

The result of mineral analysis of *Cucumis metuliferus* seeds and juice is presented in Table 1. The seeds contained higher concentrations of Mg, Zn, Cu, K and Na than the juice while the juice had higher concentrations of Ca, Al, Fe and Mn. There was no significant difference between the mineral content of the seeds and the juice.
Table 1. Mineral compositions of the seeds and juice of *Cucumis metuliferus*

| Minerals         | Concentration (ppm) | Seeds | Juice |
|------------------|---------------------|-------|-------|
| Calcium (Ca)     | 20.084a             | 23.416|       |
| Aluminium (Al)   | 0.079a              | 0.094 |       |
| Magnesium (Mg)   | 29.749              | 20.592|       |
| Zinc (Zn)        | 4.184               | 1.271 |       |
| Iron (Fe)        | 0.934               | 1.243 |       |
| Manganese (Mn)   | 0.221a              | 0.242a|       |
| Copper (Cu)      | 0.125               | 0.030 |       |
| Sodium (Na)      | 8.927               | 8.594 |       |
| Potassium (K)    | 7.594a              | 6.833a|       |

Means with the same letter are significantly different from each other (p>0.05). ND = Not detected

3.2 Heavy Metal Composition

The heavy metal contents of the seeds and the juice of *Cucumis metuliferus* are shown in Table 2. The juice contained higher significant concentrations of As, Se and Hg. Ag, Se and Hg were not detected in the seeds.

Table 2. Heavy metal compositions of the seeds and juice of *Cucumis metuliferus*

| Heavy metals | Concentration (ppm) | Seeds | Juice |
|--------------|---------------------|-------|-------|
| Arsenic (As) | 0.578a              | 20.082a|       |
| Cadmium (Cd) | 0.389               | 0.082 |       |
| Lead (Pb)    | 1.455               | 4.135 |       |
| Cobalt (Co)  | ND                  | 0.178 |       |
| Chromium (Cr)| 0.545               | 0.252 |       |
| Nickel (Ni)  | 0.288               | 0.016 |       |
| Silver (Ag)  | ND                  | 0.074 |       |
| Selenium (Se)| ND                  | 7.246 |       |
| Mercury (Hg) | ND                  | 4.609 |       |

Means with the same letter are significantly different from each other (p>0.05). ND = Not detected

3.3 Amino Acids Compositions of the Seeds and Juice of *Cucumis metuliferus*

The essential and nonessential amino acid compositions of the seeds and juice of *Cucumis metuliferus* are presented in Tables 3 and 4. The samples contained 9 essential and 9 nonessential amino acids respectively. However, the total essential amino acid (TEAA) content of the juice was higher than that of the seed while the total amino acid and total nonessential amino acids content of the seed was higher than that of the juice. There was no significant difference between the amino acid content of the seeds and the juice (p>0.05).

Table 3. Essential amino acid compositions of the seeds and juice of *Cucumis metuliferus*

| Amino acids | Concentration (g/100g) | Seeds | Juice |
|-------------|------------------------|-------|-------|
| Valine      | 4.748                  | 5.665 |       |
| Threonine   | 3.575                  | 3.691 |       |
| Isoleucine  | 2.849                  | 4.136 |       |
| Leucine     | 2.649                  | 7.079 |       |
| Lysine      | 3.854                  | 3.595 |       |
| Methionine  | 1.496                  | 1.298 |       |
| Phenylalanine| 5.755                | 3.459 |       |
| Histidine   | 4.759                  | 2.319 |       |
| Tryptophan  | 1.789                  | 1.154 |       |
| Total EAA   | 31.475                 | 32.396|       |

4. DISCUSSION

Minerals and amino acids are essential compounds needed in food to meet various nutritional needs and as aids in biochemical processes in man and animal. Minerals generally participate in enzymatic activities. They are essential for the right functioning of tissues and function as second messengers in cascade of some biochemical mechanisms [23]. The electrolytic balance of the body fluid are dependent on the adequacy of sodium (Na), potassium (K), magnesium (Mg) and zinc (Zn).

The results of the analysis of minerals from this study showed that *Cucumis metuliferus* seeds had higher concentrations of sodium (8,927...
Calcium (23.416 ppm), Aluminum (0.094 ppm), potassium (7.594 ppm), magnesium (29.749 ppm) and zinc (4.184 ppm) than the juice (8.594, 6.833, 20.592 and 1.271 ppm respectively). Sodium and potassium are principal cations of extracellular and intra-cellular fluids and aid in maintaining electrolyte balance in the body [24]. Sodium regulates fluid balance in the body and helps in the proper functioning of muscles and nerves [25]. The values obtained from this study is low for both the seed and juice when compared with the daily recommended value of 2400 mg for adults and children of 4 years and older but could supplement in meeting Na needs in diet. However, high dietary sodium is implicated in cardiovascular and renal disorders [26]. And so, sodium is not recommended for people who suffer from or are at risk of hypertension. Potassium is very important in the maintenance of the body fluid volume and osmotic equilibrium, the pH of the body, regulation of muscles and nerve irritability, control of glucose absorption and enhancement of normal retention of protein during growth [27].

High concentration of potassium in the body was reported to increase iron utilization [28] and beneficial to people taking diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid [29]. The values obtained from this study are also low compared to the recommended dietary allowance for potassium of 4700 mg per day for an adult [30]. Magnesium was the mineral found in highest concentration for both the seed and the juice. Magnesium is needed in the plasma and extracellular fluid and aids in maintaining osmotic equilibrium [31]. It prevents cardiovascular disorders and lower blood pressure in humans. Magnesium is an active co-enzyme in carbohydrate and protein metabolism [32]. Zinc is an essential element for plants and animals, but only a small increase in its level may cause interference with physiological processes [33]. It is said to be vital in protein synthesis, cellular differentiation and replication, immunity and sexual functions [34]. Impaired zinc absorption, vascular disease and cancer as well as systemic iron overload may be associated with iron toxicity in human body [35]. Zinc deficiency especially in children can lead to loss of appetite, growth retardation, weakness and stagnation of sexual growth [36]. The maximum tolerable daily intake of Zn is 0.3-1 mg/kg [37]. The zinc content of both the seeds and the juice of Cucumis metuliferus were found to be greater than the tolerable daily intake level. On the other hand, the juice had higher concentrations of Calcium (23,416 ppm), Aluminum (0.094 ppm), Iron (1.243 ppm) and Manganese (0.242 ppm) than the seeds (20.084, 0.079, 0.934 and 0.221 ppm respectively). Calcium is needed for blood clotting, bone and teeth formation as its deficiency gives rise to conditions such as rickets and calcification. When the calcium supply to the body becomes insufficient, the body on its own extracts the needed calcium from the bones. If the body continues to tear down more calcium than it replaces over a period of years the bones will become brittle. Calcium is important for prevention and control of disease and therefore contributes to the medicinal influences of the plant [38]. The concentrations of aluminum (Al) in both the seeds and juice are low and this is good as Al is involved in the biological cycle that might cause serious damage to humans and other living organisms [39]. Aluminum is generally not regarded as an essential element and its benefits are not documented. Iron is a part of the heme of haemoglobin (Hb), myoglobin, and cytochromes needed for transporting oxygen and carbondioxide [40]. Minimum daily requirement is dependent on age, sex, physiological status, and iron bioavailability, and range from 10 to 50 mg/day [35]. Established provisional maximum tolerable daily intake (PMTDI) of 0.8 mg/kg of body weight applies to iron from all sources [41]. Its deficiency leads to anaemia while excess of it beyond 45 mg/day have critical gastrointestinal side effects [42]. From the values obtained in this study, the iron content for both the seeds and the juice of Cucumis metuliferus are lower than the maximum tolerable daily intake level. Manganese is a co-enzyme in urea formation, pyruvate metabolism and the galactotransferase catalysis of connective tissue biosynthesis [40]. Manganese requirement in the body is small compared to iron with daily requirement for healthy living of 4.5 mg [43] with tolerable daily intake level of 12 mg/day.

The presence of several non-essential elements in the seeds and juice of Cucumis metuliferus such as chromium, nickel, cadmium, arsenic, lead, cobalt, silver, selenium and mercury can be attributed to contamination from the soil, water, atmosphere and chemicals used for artificial ripening. These elements are trace metals and regarded as heavy metals. Trace element is any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate [44]. They constitute significant health hazards for man and have become an area of particular concern and highest priority in environmental research [45].
Fruits are highly valued in human diet for vitamins and minerals. Eating of artificially treated fruits is very harmful and responsible for many life-threatening diseases in human beings. Heavy metals are used as materials which are always in contact with food, mainly in processing equipment, containers and household utensils and also in foils for wrapping foodstuffs [44]. They serve as safety barriers between the food and the exterior. These materials are often covered by a surface coating, which reduces the migration in foodstuffs. When they are not covered, these food contact material’s metal ions can easily migrate into the foodstuffs and therefore could either endanger human health if the total content of the metals exceeds the recommended exposure limits, or bring about an unacceptable change in the composition of the foodstuffs or a deterioration in their organoleptic characteristics [44]. The juice of *Cucumis metuliferus* fruit in this study had higher concentrations of arsenic (20.082ppm), lead (4.135 ppm), cobalt (0.178 ppm), silver (0.074 ppm), selenium (7.246 ppm) and mercury (4.609 ppm) as against the seeds with respective values of 0.578 ppm, 1.455 ppm while cobalt, silver, selenium and mercury were not detected. On the other hand, the concentrations of cadmium (0.389ppm), chromium (0.545ppm) and nickel (0.288ppm) were higher in the seeds than in the juice with respective values of 0.082ppm, 0.252ppm and 0.016ppm. Data from analysis of food and intake of the U.S. Department of Agriculture Continuing Survey of Food Intakes by individuals indicates that the safety intake of Arsenic for all age groups ranged from 0.50 to 0.81 μg/kg/day [46]. Arsenic is an established human poison. Ingestion of doses greater than 10 mg/kg/day can lead to anemia and hepatotoxicity [47]. The value of arsenic obtained from the analysis of the juice in this study is way too high and beyond the safety dose. Lead is also another poisonous compound. It induces reduced cognitive development and intellectual performance in children, cardiovascular disease and increased blood pressure in adults [48,49, 50]. According to the national standard of China on maximum levels of contaminants in foods, maximum level for lead in fruits is 0.10 mg/kg [51]. The limit set by FAO/WHO is 0.3mg/kg. The values obtained for the seeds and the juice of *Cucumis metuliferus* in this study are beyond these limits. Availability of lead in fruits may be due to the use of ripening agents or due to the high concentration of lead aerosol in the air surrounding the area resulting from emission from automobile exhaust [44]. The main sources of lead intake are foodstuffs like vegetables (up to 0.05 mg/kg), cereals and cereal products (up to 0.09 mg/kg), fruit and fruit juices as well as wine, beverages and drinking water [52]. Cobalt is regarded as a heavy metal although beneficial to human. It is a key component of cobalamin (vitamin B12), the primary biological reservoir of cobalt as an ultratrace element [53]. The minimum presence of cobalt in soils improves the health of grazing animals markedly and an uptake of 0.20 mg/kg a day is recommended for them, as they can obtain vitamin B₁₂ in no other way [54]. Cobalt is toxic because of its solubility in water [55]. Exposure to high levels results in lung and heart diseases and dermatitis. It induces a rise in hemoglobin in anemic patients with nephritis, cancer and chronic infections. The average daily intake of cobalt is estimated to be 5 to 40 μg per day (0.04 mg) [56]. The result from this study showed that cobalt was found only in the juice with a concentration of 0.178 ppm. This is above the estimated daily intake. Selenium is another heavy metal found in high concentration in the juice only. Selenium is nutritionally essential. It is a constituent of many selenoproteins that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection [57]. It is also a constituent of glutathione peroxidase which is a major scavenger of hydrogen peroxide [58,59]. Nevertheless, more than 5 mg of selenium is highly toxic as the recommended maximum daily intake is 0.45 milligram [60]. Long-term poisoning can have paralytic effects [61]. The selenium content of the juice of *Cucumis metuliferus* in this study (7.246 ppm) is far above the tolerable upper intake level and so excessive consumption may expose people to toxicity from selenium. At intakes of 4–8 mg/kg, selenium increases the copper contents of the heart, liver, and kidneys but has a detoxifying or protective effect against cadmium and mercury [62,63]. High selenium intake has also been shown to decrease sperm motility in healthy men [64] and has been related to increased incidence of some forms of cancer including pancreatic and skin cancer. Mercury, also found only in the juice is one of the most toxic elements among heavy metals and exposure to high level of this element could permanently damage the brain, kidneys and developing fetus [65]. Mercury is among the metals of most concern for human health, especially organic mercury [44]. Mercury in ambient air originates mainly from volcanic activity and industrial activity [52]. Methyl mercury is biosynthesised from inorganic...
mercury as a consequence of microbial activity [44]. The average daily intake of mercury is between 0.002-0.02 mg [52]. The maximum level for mercury in fruits is 0.01 mg/kg as reported by the national standard of China on maximum levels of contaminants in foods [51]. The value obtained from this study on the mercury concentration of the juice (4.609 ppm) is higher than the recommended daily intake. Cadmium, chromium and nickel were found only in the seed at moderate levels of 0.389, 0.545 and 0.288 ppm respectively. Cadmium and chromium are higher than the permissible limit of 0.2 -1.0 mg/kg and 0.50 mg/kg respectively in fruits [51] while nickel is below the permissible limit of 2.7 mg/kg recommended by NAFDAC. From report, cadmium accumulates in the human body and induces kidney dysfunction, skeletal damage and reproductive deficiencies [66]. The effects of cadmium on humans also include cardiovascular-toxicity and genotoxicity. Ingestion of highly contaminated foodstuffs results in acute gastrointestinal effects in form of diarrhoea and vomiting. About 5% of ingested cadmium is absorbed [52]. Chromium (III) is an essential nutrient that helps the body in absorption of sugar, protein and fat but chromium (VI) is carcinogenic [66]. Adverse health effects may arise from excessive amount of chromium (III) [67]. Deficiency of chromium results in impaired growth and disturbances in glucose, lipid and protein metabolism. Cr (IV) is also implicated in cancer [68,69,70]. Nickel is a known carcinogen [71]. Inhaled nickel carbonyl, a carcinogenic gas that results from the action of nickel with heated carbon monoxide, from cigarette smoke, car exhaust, and some industrial wastes is very toxic [72]. Nickel allergy can also cause systematic reactions [73].

Proteins of both animal and plant is made up of 20 standard amino acids. Amino acids are required for the synthesis of body protein and other important nitrogen-containing compounds, such as creatine, peptide hormones, and some neurotransmitters. Proteins and other nitrogenous compounds are continuously degraded and resynthesized. Several times more protein is turned over daily within the body than is ordinarily consumed, indicating that reutilization of amino acids is a major feature of the economy of protein metabolism. This process of recapture is not completely efficient, and some amino acids are lost by oxidative catabolism. Metabolic products of amino acids (urea, creatinine, uric acid, and other nitrogenous products) are excreted in the urine; nitrogen is also lost in feces, sweat, and other body secretions and in sloughed skin, hair, and nails. A continuous supply of dietary amino acids is required to replace these losses, even after growth has ceased.

The result from the analysis of amino acid composition of the seeds and juice of Cucumis metuliferus revealed that both contained 18 amino acids which include 9 essential and 9 nonessential amino acids as shown in Tables 3 and 4. There was no significant difference (p>0.05) between the amino acid content of both the seeds and the juice. The seeds contained higher concentrations of all the amino acids except proline, valine, threonine, isoleucine and leucine than the juice. The obtained results of amino acid composition in the seeds of Cucumis metuliferus are comparable with that reported by Karaye et al [74] and higher than the reported values of the seeds of Citrullus colocynthis fruit by [75]. The estimation of protein requirements takes into account their quantity and quality which is mainly determined by the amino acid composition particularly the essential amino acids [76]. The most abundant essential amino acid found in the seeds was phenylalanine (5.755 g/100g) while that of the juice was leucine (7.079 g/100g). On the other hand, the most abundant nonessential amino acid found in the seeds was aspartate (9.785 g/100g) and proline (7.749 g/100g) respectively. More so, aspartate was the most dominant of all the amino acids found in the study. The high level could be due to the experimental conditions that could cause the transformation of asparagine into aspartate. Glutamate was the second most abundant in both the seeds and the juice. This could also be due to rapid conversion of glutamine to glutamate. Concentration of all the nine essential amino acids found in both the seeds and the juice were all above the recommended daily intake values by WHO for a 70kg man [77,78] except leucine in the seeds (2.649g/100g) which was a little below that recommended by WHO (2.730g). However, leucine was the most abundant in the juice and of all the essential amino acids in the study. With the high amount of these essential amino acids, the fruit of Cucumis metuliferus can be recommended to meet the need of essential amino acids which must be obtained from diet for proper metabolism of nutrients. This is because foodstuffs that lack essential amino acids are poor sources of protein equivalents, as the body tends to deaminate the amino acids obtained, converting proteins into fats and carbohydrates.
Therefore, a balance of essential amino acids is necessary for a high degree of net protein utilization, which is the mass ratio of amino acids converted to proteins to amino acids supplied [79]. If one of the essential amino acids is less than needed for an individual the utilization of other amino acids will be hindered and thus protein synthesis will be less than adequate [80]. Protein deficiency has been shown to affect all the organs and systems of the body including the brain and brain function of infants and young children; the immune system, thus elevating risk of infection; gut mucosal function and permeability, which affects absorption and vulnerability to systemic disease; and kidney function [80]. Of worthy of note also is the relatively high concentration of histidine in the seed. Histidine is an essential amino acid for infants for proper growth and has been demonstrated to be required by adults recently [81,82]. Therefore, the seeds of *Cucumis metuliferus* is recommended in food supplement for growing children.

In general, the total amino acid and total nonessential amino content of the seeds were higher than that of the juice. However, the total essential amino acid content of the juice was higher although non-significantly. This shows that the juice has higher protein and fat contents which are of biological values to be used as good sources in the daily amino acid requirements for adults [83].

5. CONCLUSION

From the results of comparative assessment, *Cucumis metuliferus* seeds and juice are good sources of essential minerals, amino acids and potential sources of proteins. The mineral contents have potentials to meet the nutritional requirements of human health. The heavy metal contaminant could be through artificial treatment of the fruits. The daily intake of arsenic, cadmium, lead, mercury and chromium through fresh fruits may constitute a health hazard for consumers because the values were all above the recommended daily intake of these metals. It is therefore recommended that the use of adulterants in fruits must be strictly prohibited in order to prevent excessive build-up of these metals in the human food chain. From the result of amino acid analysis, it was found out that the seeds and juice of *Cucumis metuliferus* are good protein sources as they contain essential amino acids and nonessential amino acids and thus could be used as a food supplement. Also, amino acids are the building blocks-functional units of proteins used daily for growth and good health of growing children and adults. Therefore, this study indicates that *Cucumis metuliferus* seeds and juice are excellent sources of minerals and amino acids.

ACKNOWLEDGEMENT

The authors wish to acknowledge and thank the management and laboratory technologists of Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Nigeria for their technical support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58474