Comparative Study of the Nutritional, Anti-Nutritional and Heavy Metal Compositions of Some Indigenous and Newly Imported Rice Varieties in Nigeria

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Abstract: A comparative study of the nutritional, anti- nutritional and heavy metal compositions of one indigenous (local rice) and two selected of the newly Long grain rice (Basmati and imported rice) varieties got from different markets in Awka Anambra State were carried out. This was performed using compositional analyses of Association of Official Analytical Chemists (AOAC, 1984) and Atomic Absorption Spectrophotometer (AAS) for heavy metal evaluation. The proximate analysis showed that the rice varieties had high percentage Carbohydrate contents with 75.75% least for imported rice and 84.45% highest for Basmati rice and relatively low moisture content of 2-4.2%. All of the rice varieties studied showed low fat content of 0.4-1.0%. The ash content was very high in Basmati rice at 8.5% and lowest in imported rice at 1.0%. Local and imported rice varieties had high contents of crude fibre of 6.5% and 5.0% respectively but low in Basmati rice at 1.5%. Imported rice had an appreciable high protein content of 15.05% followed by local rice of value 8.75% whereas the protein content is very low in Basmati at 3.15%. High fibre and protein contents added more advantage to local and imported varieties. The anti-nutrients in the samples studied were very low compared to its normal intake; and so are safe for consumption. The heavy metal content of the samples studied were higher than the provisional tolerance intake regulations by WHO/FAO except for zinc content, which was within the standard. Thus from the obtained results, it was observed that the results for local rice was the best when compared with other rice varieties studied in terms of higher nutrients, less heavy metals and low anti-nutrients.

Keywords: Rice, Heavy metals, Proximate analysis, Anti-nutrients.

1. INTRODUCTION

Rice (*Oryza sativa*) is the second leading cereal crop and staple food of approximately one- half of the world population [1]. It is the only major cereal crop that is consumed almost exclusively by humans. Rice is grown in at least 114 countries (e.g. Pakistan, France, Korea, Japan, Taiwan etc.) with global production of 645 million tons. More than 90% of the world's rice is grown and consumed in Asia and it is consumed by the Asians up to 35 - 60% of calories.

Rice is grown in all the ecological and dietary zones of Nigeria, with different processing adaptation trait for ecology [2,3]. The two commonly cultivated species of rice are *Oryza sativa*, the Asian rice, grown worldwide and *Oryza glaberrima*, the African rice, grown on a limited scale in West African [4]. Also there are twenty-one wild species of genus Oryza. Nine of the wild species are tetraploid while the remaining wild species as well as the cultivated species are diploid. The cultivated species are thought to be an example of parallel evolution in crop plants.

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Rice is an important food component of the daily diet, providing carbohydrate, proteins, dietary fibers and vitamins. Epidemiological studies have indicated protective role of whole grain foods (e.g. rice) against several diseases associated with westernized societies such as type 2 diabetes [5-8], Cardiovascular diseases and certain cancers. Rice is an economic crop, which is important in household food security, ceremonies, nutritional diversification, income generation and employment. It is utilized mostly at the household level, where it is consumed as boiled rice or fried with stew or soup. Rice is a good source of insoluble fiber, which reduce the risk of bowel disorder and fights constipation. Rice is also rich in minerals but the bioavailability of these minerals is usually low due to the presence of anti-nutritional factors like phytates, trypsin inhibitor, etc. Many Nigerians prefer to consume foreign rice brand compared to any local or unrefined rice varieties. In this research, the proximate (nutritional), anti-nutritional factors and heavy metal composition of some selected indigenous and newly imported rice varieties as an index of their nutritional worth were studied.

2. MATERIALS AND METHODS

The rice samples were collected from the local market in Awka, Anambra State, South Eastern Nigeria. They were ground to fine texture and sieved

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before analysing them. These ground samples were kept in a sealed container for analysis. The nutritional (proximate analysis) and anti- nutritional contents of the rice sample as well as the presence of or absence of metals in the selected rice samples.

3.PROXIMATE (NUTRITIONAL) ANALYSIS

3.1. Moisture Content

3.1.1. Procedure

A Petri -dish was washed, and dried in an oven. Exactly 5g of the sample was weighed into the Petri-dish and weight of the Petri-dish and sample was recorded before drying. The Petri-dish and the sample were put in an the for another 30 minutes and the weight was recorded. The drying procedure was continued until a constant weight was obtained, then the moisture content was calculated.

3.1.2. Calculations

% Moisture = $W_1 – W_2$ X 100/ weight of sample

Where W_1 = weight of Petri-dish and sample before drying

W_2 = weight of Petri-dish and sample after drying

3.2. Ash Content

3.2.1. Procedures

Empty platinum crucible was washed, dried and the weight was recorded. Exactly 2g of the sample was weighed into the platinum crucible and placed in a muffle furnace at 550^0c for 3 hours. The sample was cooled in a desiccator after burning and weighed. The ash content was then calculated.

3.2.2. Calculations

% Ash = $W_1 – W_2$ X 100/ weight of sample

W_1 = weight of empty crucible in gram

W_2 = weight of crucible and sample after burning

3.3. Crude Fibre

3.3.1. Procedure

About 2g of material was defatted with petroleum ether (if the fat content is more than 10%). This was then boiled under reflux for 30mins with 200ml of a solution 1.25g of H_2SO_4 per 100ml of solution.

The solution was filtered through linen or layers of cheese cloth on a fluted funnel and were washed with boiling water until the washings are no longer acidic.

The residue was transferred to a beaker and was boiled for 30mins with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. The solution was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible. It was dried in an electric oven, weighed, incinerated, cooled and weighed again.

The loss in weight after incineration x 100 was calculated as the percentage of crude fibre.

3.3.2. Calculations

% Fibre = $W_1 – W_2$ X 100 / weight of sample

W_1 = oven dried weight

W_2 = incinerated weight

3.4. Crude Fat

3.4.1. Procedure

250ml clean boiling flask was dried in an oven at $105\text{-}110^0$c for about 30mins and was transferred into a dessicator and allowed to cool. The flask was weighed correspondingly, labeled and filled with about 300ml of petroleum ether (boiling point $40\text{-}60^0$c). 2g of sample was weighed into the extracting thimble, then the thimble was lightly plugged with cotton wool and the soxhlet apparatus were assembled and allowed to reflux for about 6hours. The thimble was removed with care and petroleum ether was collected at the top container of the set-up and was drained into a container for re-use. When flask was almost free of petroleum ether, it was removed and dried at 105^0c for 1 hour and was transferred from the oven into a dessicator and allowed to cool; then weighed.

3.4.2. Calculations

% Fat = $W_2 – W_1$ X 100/ weight of sample

W_1 = weight of flask

W_2 = weight of flask and oil

3.5. Crude Proteins [10]

3.5.1. Procedures

Exactly 2g of sample was weighed into a 30ml kjehdal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stopped and shaken). The 1g of the kjedahl

catalyst (selenium powder) was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30mins and allowed to cool. After cooling about 100ml of distilled water was added to avoid caking and then 50ml was transferred to the kjedahl distillation apparatus.

A 100ml receiver flask containing 5ml of 2% boric acid and indicator mixture containing 5 drops bromo crypsol blue and 1 drop of Methylene blue was placed under a condenser of the apparatus. Sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until 50 drops got into the receiver flask, after, which it was titrated to pink color using 0.01N hydrochloric acid.

3.5.2. Principle

The method is the digestion of sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia. This is retained in the solution as ammonium sulphate;

$$RN_2 + 2H_2SO4 \longrightarrow (NH_4)_2SO_4 + CO_2 + H_2O$$

The solution is made alkaline, and then distilled to release the ammonia;

$$(NH4)_2SO_4 + 2NaOH \longrightarrow 2NH_3 + H_2O + Na_2SO_4$$

The ammonia is treated in dilute acid;

$$3NH_3 + H_3BO_3 \longrightarrow (NH4)_3BO_3$$

and then titrated;

$$(NH_4)_3BO_3 + HCl \longrightarrow H_3BO_3 + 3NH_4Cl$$

3.5.3. Calculation

% Nitrogen = titre value (T) x atomic mass of N x normality of acid x 4

% Crude protein = % N x 6.25

6.25 = Conversion factor for protein

Normality of acid = 0.01N HCl

Note: Most proteins contain 16% nitrogen so that 16mg N = 100mg protein, 1mg Nitrogen = 6.25

3.5.4. Carbohydrate Determination (Differential Method)

100 – (% protein + %moisture + % Ash + % Fat + % Fibre)

4. ANTI- NUTRITIONAL ANALYSIS

4.1. Oxalate Determination by Titration Method

This determination involves three major steps digestion, oxalate precipitation and permanganate titration.

4.1.1. Digestion

i. 2g of sample was suspended in 190ml of distilled water in a 250ml volumetric flask.

ii. 10ml of 6cm HCl was added and the suspension digested at 1000^0C for 1 hour

iii. Cool, and then made up to 250ml mark before filtration.

4.1.2. Oxalate Precipitation

Duplicate portions of 125ml of the filtrate are measured into beakers and four drop of methyl red indicator added. This was followed by the addition of NH_4OH solution (drop wise) until the test solution changes from salmon pink color to a faint yellow color (pH of 4- 4.5), each portion was then heated to 90^0C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated 90^0C and 10ml of 5% $CaCl_2$ solution is added while being stirred constantly. After heating, it was cooled and left overnight at 25^0c. The solution was then centrifuge at 2500 rpm for 5 minutes. The supernatant was decanted and the precipitate completely dissolved in 10ml of 20% (v/v) H_2SO_4 solution.

4.1.3. Permanganate Titration

At this point, the total filtration resulting from digestion of 2g of flour was 300ml. About 125ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized $KMnO_4$ solution to a faint pink color, which persists for 30s. The calcium oxalate content was then calculated.

4.1.4. Calculation

% Oxalic acid = Titre value X Vme X DF/ME X MF

Vme = Volume of acid (titrant)

DF = Dilution fact

ME = Molarity of acid

MF = Mass of sample

4.2. Cardiac Glycosides Determination

Wang and Filled method [9] was used. To 1ml of extract was added 1ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1ml of 5% aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried an oven at 50^0c till dryness and weight of the filter paper with residue was noted. The cardiac glycoside was calculated in percentage.

4.2.1. Calculation

% Cyanogenic glycoside = $W_2 – W_1$ X 100/ weight of sample

4.3. Phytate Determination

Phytate contents were determined using the method of Young and Greaves (1940) as adapted by Lucas Markakes (1975) [9]. 2g of the sample was placed in each of the different 250ml conical flasks. Each sample was soaked in 100ml of 2% concentrated HCl for 3hr, the sample was then filtered. 50ml of each filtrate was placed in 250ml beaker and 100ml distilled water was added to each sample. 10ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (iii) chloride solution.

4.3.1. Calculation

% Phytic acid = Titre value X 0.00195 X 1.19 X 100/weight of sample.

4.4. Heamagglutinin Determination

Two gram of each of the samples were added 20ml of 0.9% NaCl and suspension shaken vigorously for 1min. the supernatant were left to stand for 1hr, the sample were then centrifuged at 2000rpm for 10min and the suspension filtered. The supernatants in each were collected and used as crude agglutination extract. Absorbance read at 420nm.

4.4.1. Calculation

Absorbance of sample/Absorbance of standard X Conc. of standard

4.5. Cyanogenic Glycoside

Acid titration method; 10g of ground sample was passed through a 20-mesh sieve, then placed in 800ml kjeldahl flask, 100ml of distilled water was added and was macerated at room temperature for 2 hours. 100ml of distilled water was added and steam distills, collecting distillate in 20ml 0.02N $AgNO_3$ before distillation adjusts appropriately, so that tip of condenser dips below surface of liquid in receiver.

When 150ml had passed over, the distillate was filtered through gooch wash receiver and gooched with little H_2O. Excess $AgNO_3$ was titrated in combined filtrate and washings with 0.02N KCN, using Fe alum indicator.

4.6. Determination of Trypsin Inhibitor

The sample was ground passed through a 50-mesh sieve. Half a gram of sample was extracted with 50ml of distilled water for 30min with mechanical shaking at a speed of 200rpm. 10mm of the sample suspension was then destabilized by adding an equal volume of the assay buffer further and vigorously shaking for 2-3min before filtering through a what No. 2 paper. The filtrate was then further diluted with water to the point where 1ml gave 30-70% trypsin inhibition. This was done to keep the relative standard deviation (RSD) of TIA measured within ±3.5%. A suitable final concentration of the sample was around 0.1mg of the sample per mm, and for heated samples, 0.5-1.5mg/ml.

4.6.1. Procedure

The reaction was run at 37^0c. Exactly 10min after adding the trypsin solution, the reaction was stopped by injecting 0.5ml of 30% acetic acid solution with a 1-ml syringe. The absorbance at A^s_{410} (sample reading), was a measure of the trypsin activity in the presence of the inhibitors. The reaction was also run in the absence of inhibitors by replacing the sample with 1ml of water. The corresponding absorbance was symbolized as A^r_{410} (reference reading). Distilled water was then used as a blank.

4.6.2. Calculation

Defining a trypsin unit as an A_{410} increase of 0.01 under the conditions of the assay, the trypsin inhibitory activity is expressed in trypsin units inhibited (TUI) per milligram of the sample and calculated as follows:

$$\frac{\mathbf{A^r_{410} - A^s_{410}}}{\mathbf{Vol.\ of\ sample\ /\ dilute\ extract}} \mathbf{\times 100}$$

A^r 410 = Absorbance of standard read at 410nm

A^s 410 = Absorbance of sample read at 410nm

Dilute extract = 50

4.7. Determination of Hydrogen Cyanide

The alkaline titration procedure was adopted. 10g of ground sample was soaked in a mixture of 200cm^3 of distilled water and 10cm^3 of orthophosphoric acid. The mixture was left overnight to release all bounded hydrocyanic acid. The mixture was distilled until 150$cm^3$of the distillate was collected. 20cm^3 of distillate was taken into a conical flask containing 40$cm^3$of distilled water 8cm^3 of 6$moldm^3$ aqueous ammonia and 2cm^3 of 5% potassium iodine solution was added. The mixture was titrated with 0.02$moldm^{-3}$ silver nitrate to faint but permanent turbidity.

4.8. Metal Composition

Here, the atomic absorption spectroscopy was utilized in the analysis of the metal composition

4.8.1. Procedure for Heavy Metal Analysis

Heavy metals analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer according to the method of APHA 1995(American Public Health Association)

4.8.2. Procedure

Two grams each of the finely ground samples were thoroughly mixed by shaking, and 100ml of it was transferred into a glass beaker of 250ml volume, to which 5ml of concentrated nitric acid was added and heated to boil untill the volume was reduced to about 15-20ml, by adding concentrated nitric acid increments of 5ml till all the residue is completely dissolved. The mixture was cooled, transferred and made up to 100ml using metal free distilled water. The sample was aspirated into the oxidizing air- acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed.

4.8.3. Sample Digestion [9]

1. Two grams(2g) each of the dried rice samples were weighed into a digestion flask and 20ml of the acetic acid added, a mixture containing (650ml concentrated HNO_3; 80ml perchloric acid; 20ml concentrated H_2SO_4) was also added.

2. Then heated in a flask until a digest is obtained.

3. This was then diluted with distilled water to the 100ml mark.

Appropriate dilutions were then made for each element and the absorbances read.

5. RESULTS AND DISCUSSIONS

The samples contained high percentage of carbohydrate having 75.75% for imported, 78.05% for local and 84.45% for Basmati rice as shown in Table **1**. Although these values are higher than the normal carbohydrate intake of 45-65% as well as the values obtained by some researchers [11,12] with 65-75% and 74.95% respectively, they are a bit lower than the values 86.81 to 90.57% reported by Ogbuagu [12] who analyzed the nutritive values and swelling capacities of some rice brands sold in local and Abuja, Nigeria. Imported rice variety had the lowest carbohydrate content. Its low carbohydrate content may be attributed to its high protein content, which is the second most abundant constituent of rice [13]. The high percentage of carbohydrate contents implies that these rice varieties are good sources of energy.

Table 1: Results of the Percentage Proximate Analysis on the Rice Grain Samples

Parameters	Local Rice	Imported Rice	Basmati Rice
Ash content %	1.50	1.00	8.50
Moisture content%	4.20	2.80	2.00
Fat content %	1.00	0.40	0.40
Crude fibre %	6.50	5.00	1.50
Protein content %	8.75	15.05	3.15
Carbohydrate %	78.05	75.75	84.45

The protein content of these rice samples was 3.15% for basmati, 8.75% for local rice and 15.05% for imported rice. Basmati variety had the lowest protein content, which may be attributed to prolonged parboiling that lowers the protein content of rice and some other environmental factors. However, local rice and imported varieties should be highly priced because of their relatively higher percentage of crude protein, this falls within the literature review value range of protein (7-14%). They are also in agreement with the literature findings [11,12,14,19] where the proximate and mineral composition of major rice varieties in Abakaliki were studied. The percentage fibre contents in the three rice samples were 1.5% for basmati, 5.0% for imported and 6.5% for local rice, these values are higher than the range values obtained by various studies [3,4,15] where the chemical nutrient composition of selected local and newly introduced rice varieties grown in Ebonyi State were analyzed. Local rice had the highest percentage of crude fibre. This high content of fibre could be said to be a good quality trait because rice is a good source of insoluble fibre, which reduces the risk

of bowel disorder and fights against constipation [**3**]. Basmati rice had the lowest fibre value. A diet low in fibre can lead to a wide range of ailments and conditions especially among the urban dwellers that consume more refined foods. Milling also decreases the fibre content of rice.

The percentage moisture content in these rice samples were 2.0% for basmati, 2.8% for imported and 4.2% for local rice, which are lower than the literature range values for moisture (7-11%). This may be as a result of hydrological status or agronomic potential [14]. This low moisture content in them implies that the three samples have the potential of long-term storage as reported by Ebuehi and Oyewole also Oko and Ugwu [4,14]. The percentage fat content of the rice samples were 0.4% for basmati, 0.4% for imported and 1% for local rice. This result is in agreement with literature range of 0.50 to 2.23% [3,15] but lower than that obtained by Gudav [13]. This low fat content is normal because excess consumption of saturated fats increases the level of cholesterol as well as causes obesity, which is a factor in the causation of disease [2]. So because of its high level of instauration (low fat content), rice lipids of these rice samples are known to have blood cholesterol lowering effect. This range is lower than that obtained by Oko and Ugwu [4]; this may be due to the degree of milling, which removes the outer layer of the grain where most of the fats are concentrated. The percentage ash content of these samples ranges from 1.0 to 8.5% which is higher than the literature range of 1.4 to 1.9% [16]. This is also higher than that obtained by Ogbuagu and Oko [3,4]. This higher content may be attributed to poor drying when heating and thereby affects the availability of minerals.

Table 2: Results of Metal Composition in the Rice Grain Samples (mg/kg)

Metals	Local Rice	Imported Rice	Basmati Rice	WHO/FAO
Arsenic	1.028	1.166	0.743	0.065
Mercury	0.488	0.589	2.011	0.003
Lead	0.314	0.126	7.278	0.026
Zinc	2.798	2.405	4.412	8.4

The above Table **2** shows the results of the heavy metal analysis carried out on the three samples. From the table it was observed that there were high content of the heavy metals studied in my the samples. The values obtained for the heavy metal composition were lower than that obtained in the literaure [5] where the heavy metals in some locally produced Rice (*Oryza sativa*) from the Northern Region of Nigeria were obtained within the ranges of 0.311-0.525mg/kg. This range together with the range of the obtained results of 0.126-7.278mg/kg for lead, mercury and arsenic are higher than the WHO/FAO (2002) [20]. Provisional tolerance intake regulations (PTWI) of 0.026mg/kg lead, 0.065mg/kg arsenic, 0.003mg/kg mercury daily. On the contrary, the range of values for Zinc, 2.405-4.412mg/kg, was lower than the required intake value for zinc (8.4mg/kg). Thus the zinc content in these rice samples studied are less toxic compared to the lead, mercury and arsenic contents which are toxic as reported by Teresa *et al*. [16]. Consequently, the public health is at risk if they base on these varieties of rice because they are not safe for consumption. This toxicity may be caused by the rate of fertilizer application and the native fertility of paddy fields.

Table 3: Results of the Anti-Nutritional Factors in the Rice Grain Samples

Parameters	Local Rice	Imported Rice	Basmati
Oxalate %	0.070	0.097	0.043
Phytate%	0.87	0.81	0.74
Cyanogenic glycoside %	1.13	1.35	1.73
Haemagglutinin mg/g	2.56	2.38	2.10
Cardiac glycoside %	1.50	0.50	1.00
Hydrogen liquid mg/HCN	5.62	5.62	7.34
Trypsin Inhibitor mg/L	4.026	4.058	4.052

The anti nutritional factors were determined and the results illustrated in Table **3** above showed that oxalate content had the range of 0.043 to 0.097%. This is lower than that obtained by Umar *et al*. [12] with 1.25%; it is also lower than that reported by Satinder *et al*. [7] with 0.44%. Phytate content had the range of 0.74-0.87%; this is a lower range of value when compared with the literature range of 3-6%. Cyanogenic glycoside had the value range of 1.13 to 1.73%, this is lower than that obtained by other studies [12] with the value of 4.10%. This portrays a less harmful effect in these rice varieties. Heamagglutinin had the value range of 2.10 to 2.56mg/g, which is much lower than the literature value of 22.85mg/g. The cardiac glycoside had the range values of 0.5 to 1.5%; this is low compared to literature values. As well, hydrogen cyanide had value range of 5.62 to 7.34mg/HCN, which is appreciably high and can be toxic to health if present in rice in such quantity. Trypsin inhibitor was found in the rice samples in the range of 4.026 to 4.058mg/l. This is still lower than that obtained by Satinder [7] with the value of 8.01mg/l. Generally, the anti-nutrients in these rice

samples were in low amount and will have low or no toxic effect on humans when consumed.

6. CONCLUSION

It was observed that the samples contain less of some nutrients and more of others; also these are all high in carbohydrate, which entails that they are good energy giving food. The anti – nutrients in these are appreciably low and therefore are less harmful to the system when taken. The heavy metal contents in these samples were higher than the normal intake limit except their contents for zinc, which were less than required (0.026mg/kg). Thus if the risk of the heavy metal toxicity can be reduced through taking dietary measures that may promote the safe metabolism or excretion of ingested heavy metals, and if we abide by the cooking rules like avoiding too much of washing while cooking which reduces the minerals and vitamins content of rice. Local rice is considered to be the best of these samples since it contained less of the heavy metals and anti-nutrients and more of nutritional factors. The population of rice consumers is increasing annually and this demands for increase in cultivation of rice. However, there are no additional lands available for rice cultivation because the areas set out for this purpose are going down in several countries due to pressures of urbanization. The federal government should look closely to this problem as well as making provisions for rice varieties with higher yield potential and stability to meet up with the challenges of increased rice production. There is also need to continuously assess the quality of new varieties being introduced into the State and always compare them with our local varieties in order not to lose the already existing varieties which may even be better than the new ones in overall merit. Lastly, people should overlook the external outlook of some rice varieties (e.g. brown rice) as they make preferences, but consider their contents.

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