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Determination of Phytochemical and Antioxidant Properties of Some Rice Varieties and Hybrids Grown in Ebonyi State, Nigeria

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Abstract
A preliminary step to investigating the phytochemical and vitamin B contents of eleven rice lines designated as IWA (IRRI – WARDA – AGRA) lines, together with two popular and most preferred varieties (FARO 44 and R8) were carried-out. The phytochemical properties investigated included flavonoid, phenol and saponin whereas, the vitamin B included thiamine and riboflavin. The analysis revealed that the flavonoid contents of some of the IWA rice lines (IWA 3, 5 and 11) compared favourably with the control samples, FARO 44 and R8 (3.0 – 3.5 %). Phenol contents of the rice lines ranged from 0.6 – 0.74 % with IWA 1 having the least value (0.06%) and IWA 11 having the highest phenol content (0.74%). The rice samples had high contents of saponin with IWA 3 having the least (30.0 mg) and IWA 11 having the highest value (130.0 mg/100g). Results indicated that IWA 11 stands out with high values for all the phytochemical contents studied, except thiamine and will, therefore, exhibit significant antioxidant properties when consumed. Results equally indicated that five IWA rice lines (IWA 1, 2, 5, 7 and 11) had riboflavin values above the threshold required for children and adults ranges which was 0.5 %.

Keywords: Rice, Phytochemical, Vitamin, Antioxidant and Varieties
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Introduction

Rice, the seed of monocot plants and one of the major sources of carbohydrate has changed from a ceremonial to a staple food in many Nigerian homes. The demand for rice has increased at a much faster rate in Nigeria than in other West African countries. For example, during the 1960's, Nigeria had the lowest per-capita annual consumption of rice in the sub-region, averaging 3 kg. Per-capita consumption levels grew significantly at 7.3% per annum, averaging 18 kg in the 1980's and 22 kg in 1995-1999. By 2008 it rose to 32 kg, with per capita consumption in the urban areas averaging 47 kg (Adejumo-Ayibiowu, 2010; Bamidele *et al*. 2010). Nigeria is the largest producer of rice in the West African sub- region. Rice production rose gradually over the years with area expansion driven by population growth and urbanization, to surpass major rice producing countries. The country produces about 2.3 million metric tonnes of rice annually but consumes about 6.5 million metric tonnes, thus running a deficit of 4.2 million tonnes. The short fall in domestic supply have given rise to a steady increase in importation up to the tune of 60 % of the total rice supply annually (Daramola, 2005). Thus, Nigeria is also the largest importer of rice in the West African sub- region.

Rice production occurs in all agro-ecological zones in Nigeria extending from the Northern to the southern zones, with most of the rice grown in the south eastern, south south and middle belt. Rice growing environments (Imolehin, 1991) include the rain-fed upland (30 %), rain-fed lowland (47 %), irrigated lowland (16 %), deep water (1 %) and mangrove swamp (5 %). Rain-fed upland, rain-fed lowland, and irrigated systems account for 97 % of the estimated 3 million metric tonnes of total rice production in Nigeria (Daramola 2005). Production is mainly by small-scale resource poor farmers with an average farm size of 1 – 2 ha and overall average yield of 1.8 tons of paddy per hectare. They depend heavily on the use of traditional technologies, which result in low productivity (Adebayo and Onu, 2008).

The constraints to rice production are varied, including low yield in farmer's field, poor water management, inadequate weed management/control, low adoption of new technologies e.g. high yielding varieties, low level of mechanization and investment, problem of pest and disease management, ageing farming population and the effects of climate change. Climate change through extreme temperatures, frequent flooding, drought and increased salinity of water supply used for irrigation in rice fields constitute factors that affect agricultural productivity (Manneh et al., 2007). Compared to other cereals, the ability of rice to tolerate drought is poor and thus drought is a limiting factor in rice production (Laffite et al., 2003). It can occur at any stage of rice development and can cause irreversible damage at the vegetative stage. Drought stress during vegetative stage significantly reduces plant height, induce leaf rolling and prolong the vegetative phase and delay flowering even after drought stress has been relieved (Ubi et al., 2011). These ultimately affect total grain yield. Drought stress during the reproductive stages can also result in significant reductions of grain yield. This therefore emphasized the need to identify efficient and reliable ways for using available water even during stress periods.

One major option was the genetic improvement of crops for drought stress tolerance. Research at Ebonyi State University focused on "breeding for high yielding stable drought tolerance and provision of quality seeds of rice for poor resource farmers in Nigeria" (Efisue et al., 2009). The project was an Alliance for Green Revolution in Africa (AGRA)/Rockefeller Foundation project that was domiciled at the Biotechnology Research & Development Centre of the University from 2007 - 2010. As an outcome of the project, eleven rainfed lowland rice lines (identified as IWA 1 – IWA 11) and some upland lines were selected in a participatory varietal selection (PVS) with smallholder farmers. On-station yield evaluation trials indicated 45 % yield advantage of these lines over farmers' yield (Efisue et al., 2009). Three of the lines are presently undergoing the processes for official release as new varieties.

It is known that rice is one of the grains with second highest worldwide production after wheat, producing more than one fifth of the calories consumed by human species (IRRI, 1997). It contains carbohydrate, mineral, vitamins, protein, fat, and fiber. Vitamin B, precisely thiamine is the beriberi preventing factor, first isolated from rice polishing (Okwu, 2004). It is a water soluble, fairly stable vitamin with a faint yeast-like odour and salty nut-like taste whose deficiency can lead to indigestion, constipation, cardiac failure, primary fibromyalgia, etc. Vitamin B_2 also called Riboflavin is a yellow - green fluorescent pigments that form yellowish brown needle - like crystals. It plays essential role in tissue building and equally serves as coenzyme. Most of the constituents of rice, except carbohydrate, are believed to be reduced by milling. However, there is still no report of any clinical manifestation of deficiency among persons whose diet is dependent majorly on rice calories (IRRI, 1997).

Several plant species have medicinal values and are used by indigenous people for curing or the prevention of diseases. Some of these plants have anti-nutritional properties that may be toxic or could impair absorption of essential nutrients, often unknown to the local people. Yet some plants contain phytochemicals that exhibit biological effects as a result of their antioxidant properties – neutralising toxic substances and enhancing absorption of essential nutrients. The presence of phytochemicals and vitamins in dietary intake would result in good health conditions of the consumers and many health related diseases could be prevented.

In this experiment, the vitamin (B_1 & B_2) and some phytochemical constituents of IWA rice genotypes was studied. Data generated from the rice genotypes, in addition to their high yielding and resistant capabilities will go a long way in encouraging consumers to adopt them based on their advantages over existing varieties and/or age long landraces of rice in cultivation in the study area. They will also be presented as passport data to support the request for their release as new varieties. Moreover, knowledge of the preliminary phytochemical contents/profile will provide a clue to the antioxidant properties of these new rice genotypes with a view to appreciating their potentials in promoting health of the consumers. Thus, the objectives of the study include: To determine the phytochemical constituents of some rice genotypes or hybrids. To determine the vitamin constituents of the different rice genotypes.

Materials and Methods

Sample Collection: Eleven IWA rice genotypes, IWA 1 - IWA 11 were collected from Biotechnology Research and Development Centre of Ebonyi State University (EBSU), Abakaliki, Nigeria. On-station yield evaluation trials of these genotypes had indicated 45 % yield advantage over farmers' yield (Efisue et al., 2009). Selection, dissemination and adoption of the rice genotypes may translate to income generation for farmers. The controls were Faro 44, an officially released variety and R8, a popular cultivar collected from Ekpomaka in Ikwo Local Government Area of Ebonyi State, Nigeria.

Sample Preparation: The rice samples were winnowed to remove chaffs and other debris. Thereafter, the unparboiled dehusked rice seeds were pulverized using mortar and pestle into near powdered form and were sieved using 0.5 mm sieve. The husks were discarded while the sifted rice samples were stored in sterile containers in readiness for analysis. The experiment was conducted at the Biotechnology Research & Development Centre Laboratory, Presco campus, Ebonyi State University, Abakaliki.

Phytochemical Determination

Determination of flavoniod: The flavoniod content of the leaves of the sample was determined by the gravimetric methods of Harborne (1973). About 10g of the sifted rice sample was used for this analysis. Flavonoid was extracted repeatedly with 10 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was later transferred into a weighed crucible and evaporated to dryness over a water bath and then re-weighed. Weight of flavonoid was determined and expressed as a percentage of the sample weight analyzed using the formula below:

$$\% \text{ Flavonoid} = W_2 - W_1 \quad X \quad 100 / W_1$$

Where,
W = Weight of rice
W_1 = Weight of empty filter paper
W_2 = Weight of paper plus precipitate

Determination of Phenol: The phenolics in the sample were isolated according to the method of Shahidi and Naczk (1989). One gramme, of the sample was extracted thrice with 10ml of 70% (v/v) aqueous acetone at room temperature (30 ± 2^0C). This was centrifuged at 10,000 g for 10 min. The supernatant was collected, combined and evaporated to dryness at 30 ^{0}C under vacuum. The extracted phenolics were then dissolved in 25 ml methanol and re-centrifuged. To 0.5 ml of the methanolic solution was added 0.5 ml of Folin-Denis reagent (Folin-Denis reagent: To 750 ml water, 100 g sodium tungstate and 20 g phosphomolybdic acid were added in a 2l standard flask. Thereafter, 50 ml orthophosphoric acid was added and the mixture refluxed for 2 hrs. The mixture was allowed to stand and made up to 1 litre. The solution is stored in the dark prior to use) followed by the addition of 1 ml sodium carbonate and 8 ml of deionised water. The mixture was gently swirled and the mixture allowed standing for 45 min to allow for colour development. The absorbance was measured in a colorimeter at 725 nm, using the method of Swain and Hillis (1959). *Trans*-sipanic acid was used to prepare the standard calibration curve where the concentration was extrapolated.

Determination of Saponins: The total saponin content was determined using the spectrophotometric procedure described by Baccou et al. (1977). To 0.5 g defatted ground seed samples, in a screw-capped centrifuge tube were added 10ml of 80 % aqueous methanol. The tubes were tightly capped and the contents were stirred overnight using a magnetic stirrer. The tubes were centrifuged at 3000 g for 10 min at room temperature and the supernatants were collected in 25 ml volumetric flasks. The residue was washed thrice with 5 ml of 80 % aqueous methanol. Aliquots of the samples from the flasks were used for saponin determination. Diosgenin was used as the standard.

Determination of Thiamine: The Official Method of Analysis (AOAC, 1990) was used in determination of thiamine. About 5 g of the sample was homogenized with ethanolic sodium hydroxide (50 ml). This was filtered into 100 ml flask, 10 ml of the filtrate was pipette and the colour developed by addition of 10ml of potassium dichromate and read in spectrophotometer at 360 nm. A blank sample was prepared. The colour developed and read at the same wavelength.

Determination of Riboflavin: The official method of analysis (AOAC, 1990) was used in determination of riboflavin. 5 g of the sample was extracted with 100 ml of 50 % ethanol solution and shaken for one hour; this was filtered into a 100 ml flask. Ten Milliliter (10 ml) of the extract was pipette into 50 ml volumetric flask. 10 ml of 50% potassium permanganate and 10 ml of 30 % H_2O_2 were added and the setup allowed standing over a hot water bath for about 30 minutes. 2 ml of 40

% sodium sulphate was added. The solution was made up to the 50 ml mark and the absorbance measured at 510 nm using a Spectrophotometer.

Results and Discussion

The importance of rice to human species cannot be over emphasized, as it is the major staple food for millions of people in the world today. In this experiment, IWA 11 and R8 were found to be rich in flavonoid. IWA 3, IWA 5 and FARO 44 had same value for flavonoid (3.0 %), while IWA 7 and 10 had the lowest flavonoid content (0.5 %, Fig 1). According to Tapas e*t al*. (2008), flavonoids are typical phenolic compounds that can act as potent antioxidants and metal chelators. They equally possess anti-inflammatory, anti-allergic, hapatoprotective, anti-thrombotic, anti-viral, and anti-carcinogenic activities.

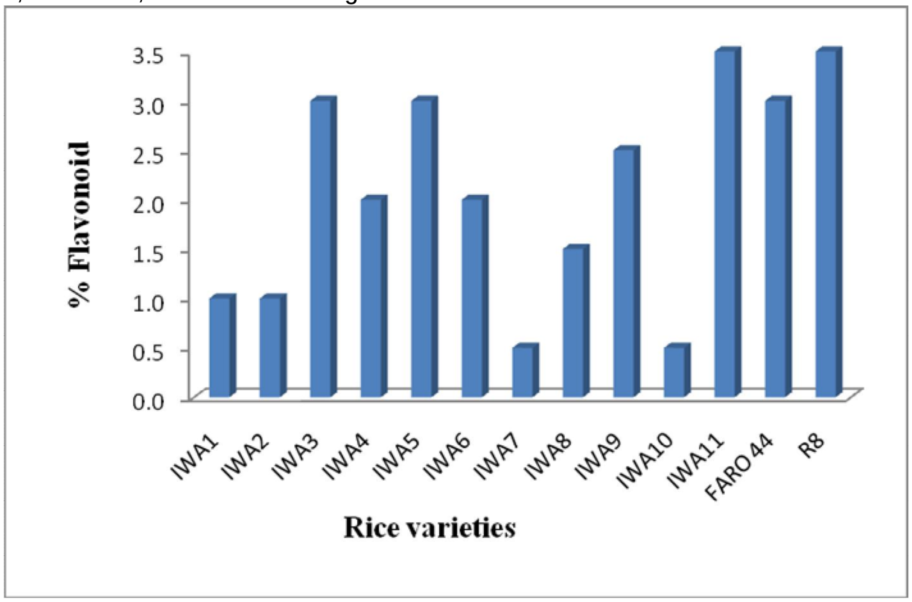

Fig 1: Flavonoid Content of Rice Varieties

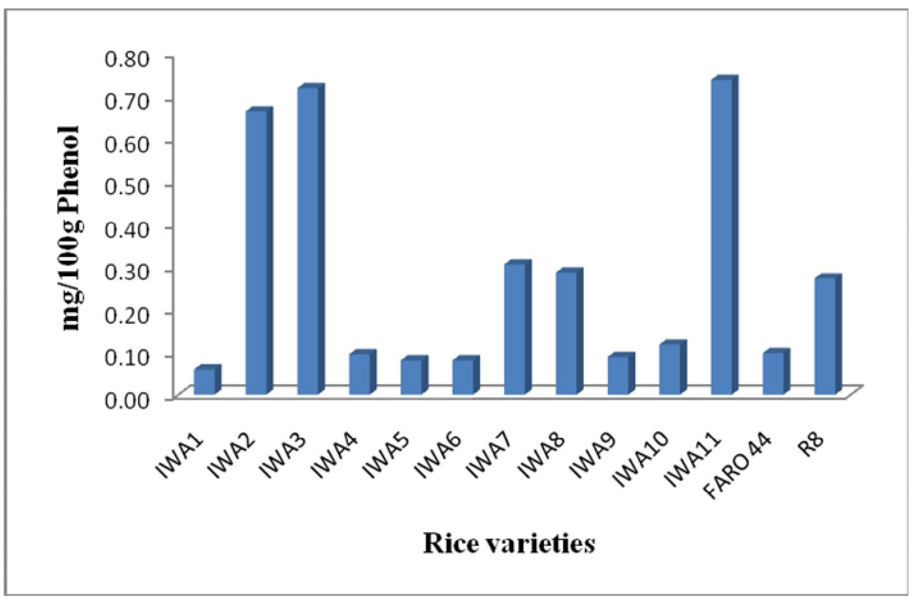

Fig 2: Phenol Content of Rice Varieties

This indicates that the new rice lines (IWA 3, 5, and 11) as well as the controls (FARO 44 and R8) can provide antioxidant properties. The high quantity of flavonoid in these rice genotypes show that they are good free radical scavenger, and will help in preventing oxidative cell damage, and protect against all stages of carcinogen in individuals whose diet is majorly on it (Okwute and Yakubu, 1998).

The result showed higher content of phenol in IWA 11, 3 and 2 (0.74, 0.72 and 0.67 mg/100g, respectively) compared to the control varieties, FARO 44 and R8 (0.10 and 0.27 mg/100g) Conversely, phenol content in IWA 1, 5, 6, and 9, in that order were lower than that of FARO 44 (Fig 2). High content of phenol in IWA 11, 3 and 2 more than the control varieties indicate that they are good antimicrobial agents. This is because phenols and phenolic compounds have been extensively used in disinfectant and remain the standard with which other bactericides are compared (Close and McArthur, 2002). High content of phenol also suggested that these varieties could act as anti- inflammatory, anti- clothing antioxidant, immune enhancers and hormone modulator (Elangovan *et al.*, 1994). Again, the present experiment indicated that IWA 11 and 3, as well as R8 are candidate varieties when flavonoids and phenols are sought in rice varieties.

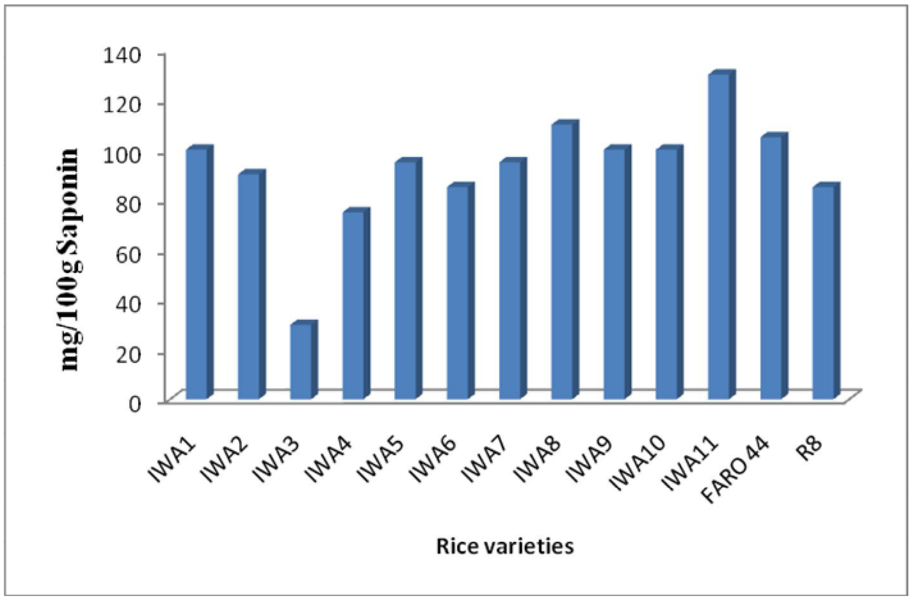

Fig 3: Saponin Content of Rice Varieties

Saponins are a class of natural products which are structurally constructed of aglycone and sugars. They exhibit a variety of biological activities, especially for various specific saponins, including their uses as anti-inflammatory, hypocholesterolemic and immune-stimulating whose properties are widely recognized and commercially utilized (Tamura et al., 2012). They are also implicated in erythrocyte haemolysis, depressed nutrient absorption and bile acid metabolism (Cheeke, 1996). Result indicated higher content of saponin in IWA 11 (130mg/100g) and IWA 8 (110 mg/100g) compared to the controls (FARO 44 and R8, Fig 5). IWA 1, 9 and 10 also had high amounts of saponin. High quantity of saponin found in IWA 11 and 8 more than the controls and other IWA rice lines studied may be suggestive of their therapeutical significance, since some of the general characteristics of saponins include formation of foams in aqueous solution, haemolytic activities and cholesterol binding properties (Hirano *et al.,* 1994; Negi, et al., 2013). Conversely, the very low content of saponin in IWA 3 should also be noted when choice of rice genotypes for consumption and release as new varieties, for specific purposes, are being made.

Vitamins are also important in the body since their deficiencies adversely affect the metabolism of the body. Results indicated highest riboflavin content for one of the control varieties, FARO 44 (0.59 mg/100g). This was closely followed by IWA I, 2, 11, 5 and 7, respectively (Fig 4). The lowest amount of riboflavin was recorded for IWA 10 and R 8 (0.31 mg/100g and 0.38 mg/100g, respectively). High quantity of riboflavin in FARO 44 more than the IWA lines suggested absence of identifiable disease or clinical manifestation and one is apt therefore, to underestimate its importance. Riboflavin is present in virtually all-living cells and it has an essential role in oxidative mechanisms. It exists in food as free compound or as part of coenzyme, Flavin Adenine Dinucleotide (FDA) and Flavin mononucleotide. These coenzymes participate in various enzyme systems such as dehydrogenesis and oxidases and as such are concerned with energy metabolism (Okaka et al., 2002). Recommended intake of riboflavin for children and adults ranges from 0.5 – 1.8 mg/day (FAO, 1988). The result indicated that the 5 IWA rice lines (IWA 1, 2, 5, 7 and 11) and FARO 44 are good sources of riboflavin and diets based on them may prevent the development of glossities.

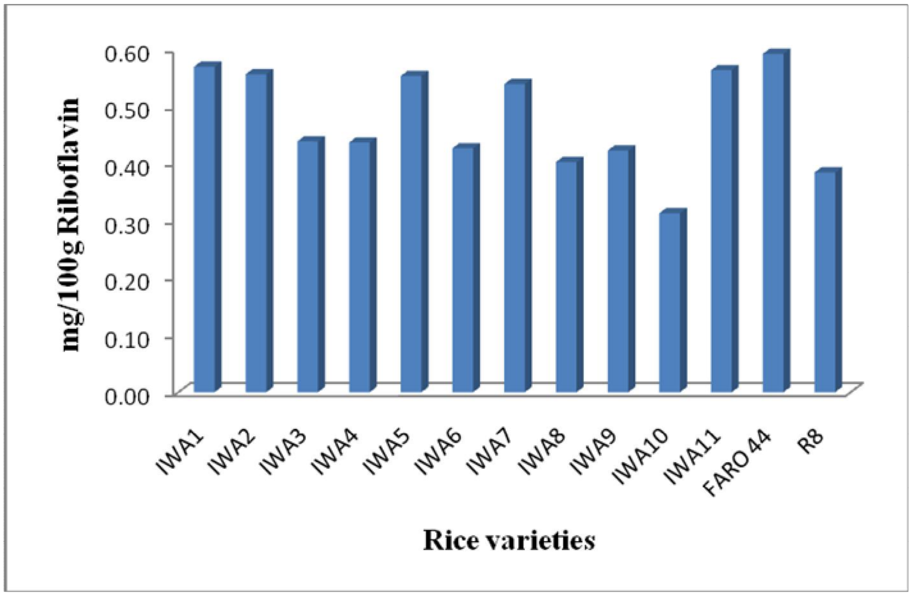

Fig 4: Riboflavin Content of Rice Varieties

The result of the thiamine contents indicated that IWA 5 and 7 had slightly higher thiamine content (0.03 mg/100g) compared to FARO 44 and R8 (0.02 mg/100g, Fig 5). Thiamine functions as a coenzyme involved in carbohydrate metabolism and nerve functions and the recommended dietary allowance (RDA) for average daily intake ranges from 1.1 – 1.5 mg/day for adults (Alaimo, 1994).

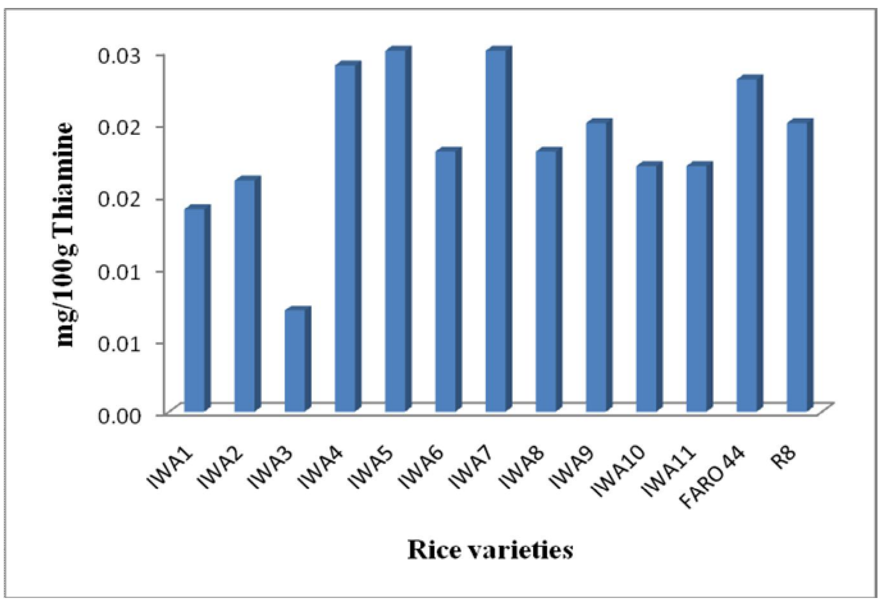

Fig 5: Thiamine Content of Rice Varieties

The levels of thiamine found in these rice samples will still reduce with processing as heat treatments are destructive of thiamine. These results showed that due to the low content of thiamine compared to the RDA for average daily intake, the IWA lines may not be active in preventing the disease beriberi. Average daily intake within the recommended daily allowance is necessary to ensure participation as coenzyme in the oxidation of glucose in the body to enable body cells obtain energy from food and to promote good appetite and digestion.

Conclusion

These improved varieties of rice are good sources of phytochemicals and some B – vitamins. They could act as antioxidants and consumption of the whole grain will result in good health conditions of the consumers and many health related diseases will be prevented. However, more breeding efforts may be required to raise the level of thiamine in candidate varieties.

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