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# Analysis of primary metabolites, inorganic phosphate, selected heavy metals and radioactive contamination in imported rice in Bahrain

***Fadheela Al-Salman[(a)], Ali Ali Redha[(a)]*, Zainab Ali[(a)], Zainab Mohammed[(a)], Mohammed Ebrahim[(a)], Zahra Ilyas[(b,c)], Sayed Adnan Hussain[(d)]***

[(a)] *Department of Chemistry, College of Science, University of Bahrain, Sakhir Campus, Zallaq P.O. Box 32038, Kingdom of Bahrain*
[(b)] *Department of Biology, College of Science, University of Bahrain, Sakhir Campus, Zallaq P.O. Box 32038, Kingdom of Bahrain*
[(c)] *Department of Laboratory, Bahrain Specialist Hospital, Juffair, P.O. Box: 10588, Kingdom of Bahrain*
[(d)] *Department of Physics, College of Science, University of Bahrain, Sakhir Campus, Zallaq P.O. Box 32038, Kingdom of Bahrain*

** Corresponding author:*
*ali96chem@gmail.com*
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## Abstract

Rice plays an important role in the diet of Bahraini people. Rice is usually imported from different countries to Bahrain, as Bahrain does not produce rice. The sources of rice and their growth conditions vary from a country to another, this directly affects the quality of rice and thus it is necessary to analyse the rice content as it has a direct impact on the society's diet. In the present study, the protein content, carbohydrate content, inorganic phosphate content, selected heavy metals (Al, Cu, Fe, Mn, and Zn) content of 8 rice samples imported in Bahrain were analysed. In addition, the gamma radiation activity concentration of all samples was measured. The rice samples analysed were glutinous rice (from Thailand), long grain parboiled rice (from Thailand), Basmati rice (from Pakistan), Punjabi basmati rice (from Pakistan), brown rice (from U.S.A.), white pudding rice (from U.K.), jasmine rice (from Italy), and organic white rice (from Slovenia). The highest protein content and inorganic phosphate content was determined in Basmati rice from Pakistan. The highest carbohydrate content and Al content was determined in long grain parboiled rice from Thailand. Brown rice from the U.S.A. had the highest Cu, Mn, and Zn content, while the highest Fe content was determined in Jasmine rice from Italy. The lowest radioactive contaminated rice was the organic white rice from Slovenia.

***Keywords:*** ***Rice primary metabolites; rice protein; rice carbohydrates; rice inorganic phosphate; heavy metals in rice; gamma radiation in rice***

## 1. Introduction

Rice (*Oryza sativa*) is an important crop which is grown in >100 countries, with 90% of total global production from Asia. It is a staple food for more than half of world's population, with more than 110,000 cultivated varieties characterized by genetics, landraces and morphology that vary in quality and nutritional content **[1].** The quality and nutrient profiles of rice vary in grain length, colour, thickness, stickiness, aroma, and growing conditions as well as production. Rice can be generally categorized as white or brown after harvesting and processing. Generally, it is consumed in its milled form, in which outer layer consisting of pericarp, tegmen and aleurone layers are removed **[2,3].** Macro and micronutrients make a major proportion of the rice grain, that tend to accumulate in outer aleurone layer. Due to a major staple, it is seen as an efficient mechanism for delivering both macro and micronutrients particularly for the poor who do not have ample access to diversified diets **[4].** Different layers of rice seed and the embryo consist different amounts macro and micronutrients. The highest amount of nutrients found in bran are dietary fibre, minerals, and vitamin B, which are lowest in the aleurone layers. the rice endosperm is rich in carbohydrate and contains a fair amount of digestible protein, with an amino acid profile which compares favourably to other grains **[5].**

To determine the nutritional value of rice, grain protein content is an important component. By improving protein content in rice, would help to enhance its nutritional profile to establish healthy lifestyles. Amino acids are the basic component of proteins, which are found to be imbalance in rice, thus lacking few essential amino acids. Lysine, threonine, and methionine are among the most critical and are limiting factors in rice grain for human nutrition as these are synthesised through specific biochemical pathways **[6].** Like cereals, rice is primarily composed of carbohydrates in form of starch that makes up almost 80% of its total dry weight. The importance of carbohydrates is that it affects the digestion of rice in human body. Carbs are major source of glucose that provide energy for many cellular chemical reactions. The relative ability of carbohydrates in food such as rice to raise blood sugar levels after eating is measured by determining Glycemic Index (GI). White rice has GI of 72, are easily digested and absorbed by the body which can result in fluctuations in blood sugar level. As compared to brown rice, which has the GI of 50, have slow rates of digestion and absorption, causing a gradual and sustained release of sugar into the blood, which is beneficial to health and reduces the chances of developing Type II diabetes **[7,8].**

In cereal crops such as rice, phytic acid (PA), or *myo*-inositol 1,2,3,4,5,6-hexakisphosphate, is the primary phosphorous (P) storage molecule. It accounts for 65-85% of total phosphate content. PA strongly chelates with mineral cations such as potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), and zinc (Zn) due to its strong negative polarity **[9].** Thus, provide the pathways to fulfil the ions need of the human body. Heavy metals from natural and anthropogenic sources tend to accumulate in soil that leak to water bodies, taken up by the plants are the consequences represent important environmental contamination problems **[10].** Study done by Changping et al., 2019 **[11],** found that heavy metal levels in soil decreased with increasing soil pH, while rice shoots accumulated heavy metals more readily under low soil pH conditions. The non-carcinogenic hazard quotients (HQ) of heavy metals show that health risks for humans were primarily due to

Pb and As. Furthermore, cancer risk (Risk) results suggested that ~76% and ~15.7% of cancer risk was caused by Cd and As levels, respectively. Decreasing soil pH enhanced the non-carcinogenic and carcinogenic health risks for the human body.

Nuclear contamination occurs as result of either burying unwanted unclear elements for decay or through destruction of nuclear power plant due to natural disaster such as Fukushima Dai-ichi Nuclear Power Plant (FDNPP) accident in March 2011. Nuclear contamination has demonstrably harmful effects on plant life and may shorten the lives of individual plants and animals **[12].** Study done by Wen-Chieh, 2005 **[13],** indicated that gamma irradiation has been proven to be effective in reducing microbial growth and increasing the shelf life of perishable foods. It was found that exposure to gamma radiation doses below 3 kGy on rice were found to have no effects on fat, starch, and protein content. Thus, gamma irradiation could shorten the indica rice aging time and improve the processing stability and quality of rice products.

Bahrain is an archipelago made up of 51 natural islands and an additional 33 artificial islands, centred around Bahrain Island which makes up around 83 percent of the country's landmass. Being tropical with less rain fall, dependent on imported rice. Therefore, the present study aims to analyse the primary metabolites such as protein and carbohydrate content, inorganic phosphate, selected heavy metals and radioactive contamination in imported rice in Bahrain.

## 2. Materials and methods

### *2.1. Sample collection*

Eight rice samples imported in Bahrain from different countries were bought and studied, the rice sample were: glutinous rice (from Thailand), long grain parboiled rice (from Thailand), Basmati rice (from Pakistan), Punjabi basmati rice (from Pakistan), brown rice (from U.S.A.), white pudding rice (from U.K.), jasmine rice (from Italy), and organic white rice (from Slovenia).

### *2.2. Estimation of protein content*

Protein content of rice samples was estimated by modified Lowry spectrophotometric method [14]. An amount of 0.01 g of rice was digested with 10 mL concentrated sodium hydroxide and heated in hot water bath at 90 °C for 10 min. After 10 min, the samples were removed and allowed to cool down at room temperature. To 1 mL of rice extract 5 mL of reagent A (containing 50 mL of 0.02 g/mL sodium carbonate and 1 mL of a solution mixture containing 0.01 g/mL sodium tartarate and 0.005 g/mL copper (II) sulphate pentahydrate) was added, mixed and allowed to stand for 10 min at room temperature. Then, 0.5 mL of diluted Folin-Ciocalteu reagent (1:16 v/v – in water) was added and allowed to stand for 30 min at room temperature. Finally, the absorbance of each reaction mixture was measured at 550 nm using AquaMate 800 UV/VIS spectrophotometer. The protein content of rice samples was determined using albumin standard curve. Egg albumin stock solution was prepared by dissolving 0.109 g of egg albumin in 100 mL saline water (prepared by dissolving 9 g of sodium chloride in 100 ml deionized water). The stock solution was diluted by serial dilution to prepare 500, 400, 300, 200, 100, and 50 μg/mL albumin standard solutions which were treated as rice extract samples to construct a standard curve.

### *2.3. Estimation of carbohydrate content*

Carbohydrate content of rice samples was estimated according to Anthrone method. An amount of 0.03 g of rice powder was dissolved in 10 mL of saline solution. A 0.1 mL aliquot of the rice solution was diluted to 1 mL using distilled water. To each sample, 5 mL of cold anthrone reagent (prepared by dissolving 0.2 g of anthrone in 5 mL absolute ethanol, followed by dilution to 100 mL with concentrated sulphuric acid in the fume hood) was added and mixed rapidly by continues swirling in ice water bath for 5 min. Finally, the absorbance of each reaction mixture was measured at 625 nm using AquaMate 800 UV/VIS spectrophotometer. The carbohydrate content of rice samples was determined using glucose standard curve. To prepare the stock solution of glucose, an amount of 0.01 g of glucose powder was dissolved in 100 mL distilled water. The stock solution was diluted by serial dilution to prepare 100, 80, 60, 40, 30, 20, and 10 μg/mL glucose standard solutions which were treated as rice extract samples to construct a standard curve.

***2.4. Estimation of inorganic phosphate content***

The inorganic phosphate content of rice samples was determined according to Al-Salman et al. [15]. The assay is based on the formation of a blue colour due to the reduction of phosphomolybdic heteropoly acid in the presence of mentol (reducing agent). Mentol, p-methyl aminophenol sulphate, reduces the anion from $[PMo1_2O_{40}]^{3-}$ to $[PMo_{12}O_{40}]^{7-}$ and the kegging structure changes from the α form to the blue β-keggin structure. The absorbance of the sample is directly proportional to the concentration of inorganic phosphate in the sample, according to Beer-Lambert law. Thus, based on this, the absorbance of reaction mixture was measured at 880 nm using AquaMate 800 UV/VIS spectrophotometer and the concentration was calculated using the standard curve of inorganic phosphate prepared using standard solutions treated as test samples.

***2.5. Determination of selected heavy metals***

The concentration of Al, Cu, Fe, Mn, and Zn in rice extracts were determined by inductively coupled plasma-mass spectrometry (ICP-MS) analysis. The ICP-MS was equipped with a micro-mist nebulizer, chiller (20 °C and 55 pis), argon gas at 20 pis, and cooling water (-40 °C). Hydrogen gas was used as a collision cell gas to avoid any interferences. To prepare the rice extract, 0.5 g of rice was digested with 7 mL concentrated nitric acid (70%) and 43 mL distilled water on a hot plate for 20 min at 150 °C. The rice solutions were filtered through 20–25 μm diameter pore filter paper of 110 mm diameter (Whatman) and diluted to 100 mL with distilled water. Triplicate set of each rice sample extract was prepared for ICP-MS analysis. The instrument was calibrated with standard solutions of the selected heavy metals.

***2.6. Analysis of radioactive contamination***

Gamma radiation contamination was measured in rice samples. An amount of 100 g of each rice sample was crushed into fine homogenous size and placed directly over Ortec-905 Scintillator Detector; operated at 865 V inside lead shields. The scintillator detector is of NaI (Tl) crystal and interfaced to a PC-computer equipped with Gamma Vision programme served as a multi-channel analyser. A background measurement (including electronic noise, cosmic radiation, and radiation in the vicinity of detector) was obtained in the area surrounding the detector, and then the radioactivity of each sample was measured. Each measurement lasted for a time duration of 14400 s (4 hours). The results were reported as activity concentration (Bq/kg).

## 3. Results and discussion

### 3.1 Physicochemical properties

The protein content of the eight rice samples was determined, which ranged between 217-620 mg albumin/ g rice as shown and illustrated in Table 1 and Figure 1, respectively. Basmati rice from Pakistan contained the highest protein content, followed by Punjabi basmati rice from Pakistan, Thai glutinous rice from Thailand, Jasmine rice from Italy and brown rice from U.S.A, organic white rice from Slovenia, white pudding rice from U.K., and long grain parboiled rice from Thailand. Pakistani basmati rice had a higher protein content compared to other types of rice. A study done by SUN, HOU, FENG and WANG, 2008 [16], found the protein content of rice samples ranged from 5 g/100 g to 13 g/100 g using digital chromatic method.

**Table 1.** Protein content of rice samples. The values are reported as mean of 3 replicates ± standard deviation.

| Rice sample | Country of import | Protein content (mg albumin/ g rice) |
|---|---|---|
| Thai glutinous rice | Thailand | 465 ±2 |
| Long grain parboiled rice | Thailand | 217 ±3 |
| Basmati rice | Pakistan | 620 ±2 |
| Punjabi basmati rice | Pakistan | 604 ±4 |
| Brown rice | U.S.A. | 434 ±2 |
| White pudding rice | U.K. | 225 ±1 |
| Jasmine rice | Italy | 434 ±0 |
| Organic white rice | Slovenia | 341 ±1 |

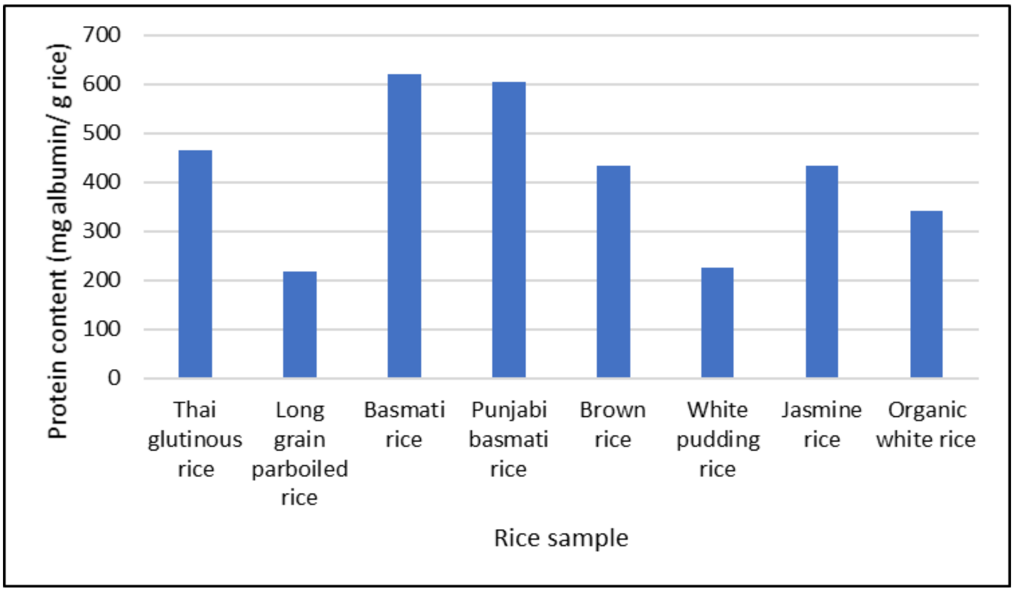


**Figure 1**. Protein content of rice sample

*3.2. Carbohydrate content*

The carbohydrate content of the eight rice samples was determined. The carbohydrate content ranged between 6.45-12.86 mg glucose/g rice as shown and illustrated in Table 2 and Figure 2, respectively. The highest carbohydrate content was determined in long grain parboiled rice from Thailand, followed by basmati rice from Pakistan, jasmine rice from Italy, Punjabi basmati rice from Pakistan, organic white rice from Slovenia, Thai glutinous rice from Thailand, brown rice from U.S.A., and the lowest carbohydrate content was determined in white pudding rice from the U.K. Since diabetes patients need to control the carbohydrate content of their diet, it could be suggested that white pudding rice and brown rice from the U.K. and U.S.A., respectively, are relatively good options for those patients. A study done by Suman and Boora, Pinky, 2016 **[17]** on basmati rice. The results reveal that, the total starch content in raw sample were 77.35g/100g.

**Table 2**. Carbohydrate content of rice samples. The values are reported as mean of 3 replicates ± standard deviation.

| Rice sample | Country of import | Carbohydrate content (mg glucose/ g rice) |
|---|---|---|
| Thai glutinous rice | Thailand | 9.16 ±0.21 |
| Long grain parboiled rice | Thailand | 12.86 ±0.15 |
| Basmati rice | Pakistan | 11.84 ±0.17 |
| Punjabi basmati rice | Pakistan | 10.96 ±0.09 |
| Brown rice | U.S.A. | 7.69 ±0.01 |
| White pudding rice | U.K. | 6.45 ±0.00 |
| Jasmine rice | Italy | 11.43 ±0.13 |
| Organic white rice | Slovenia | 10.64 ±0.06 |

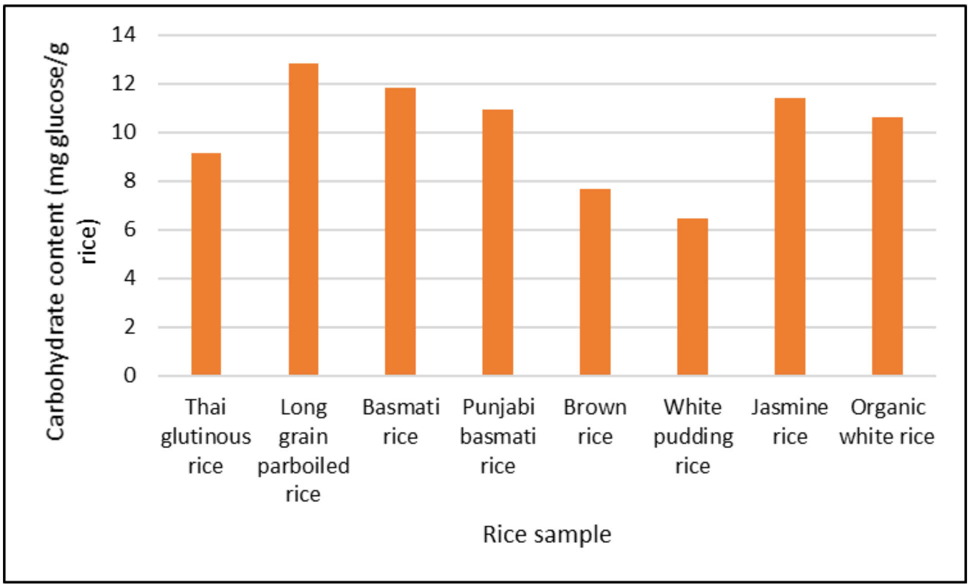


**Figure 2.** Carbohydrate content of rice samples.

*3.3. Inorganic phosphate content*

The inorganic phosphate content of the rice samples was determined, the phosphate content ranged between 0.839-1.945 mg/L, the range is relatively lower than that of protein content and carbohydrate content determined earlier as shown and illustrated in Table 3 and Figure 3, respectively. The highest amount of inorganic phosphate was determined in basmati rice from Pakistan, followed by brown rice from the U.S.A, Punjabi basmati rice from Pakistan, Thai glutinous rice from Thailand, long grain parboiled rice from Thailand, organic white rice from Slovenia, white pudding rice from the U.K., and jasmine rice from Italy. Similarly, study done by Naqqiuddin *et al.*, 2016 **[18]** found that the mean value of total phosphorus was 1.0477±0.053 mg/gDW in white pudding rice.

**Table 3**. Inorganic phosphate content of rice samples. The values are reported as mean of 3 replicates ± standard deviation.

| Rice sample | Country of import | Inorganic phosphate content (mg/L) |
|---|---|---|
| Thai glutinous rice | Thailand | 1.650 ±0.109 |
| Long grain parboiled rice | Thailand | 1.007 ±0.086 |
| Basmati rice | Pakistan | 1.945 ±0.166 |
| Punjabi basmati rice | Pakistan | 1.800 ±0.188 |
| Brown rice | U.S.A. | 1.813 ±0.136 |
| White pudding rice | U.K. | 0.850 ±0.149 |
| Jasmine rice | Italy | 0.839 ±0.139 |
| Organic white rice | Slovenia | 0.976 ±0.153 |

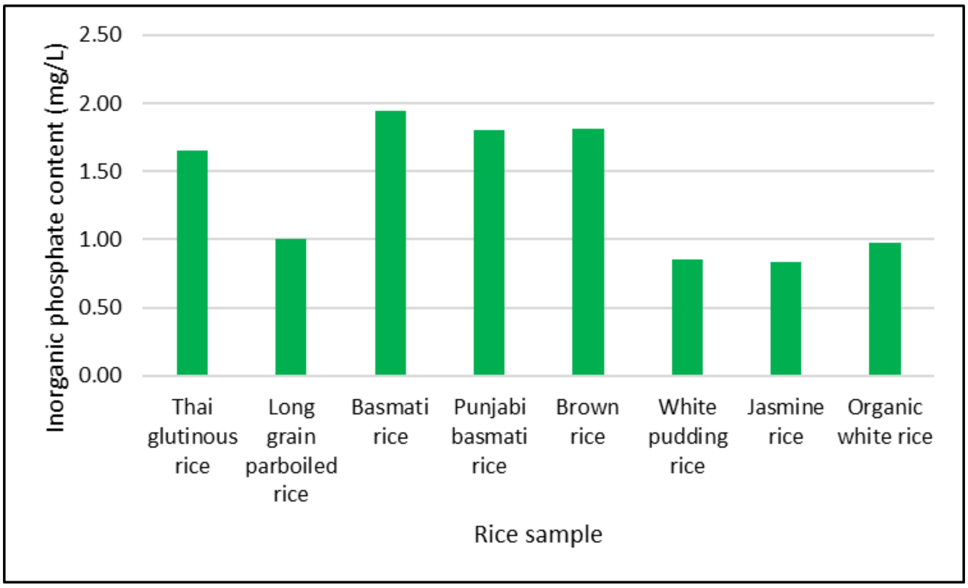


**Figure 3**. Inorganic phosphate content of rice samples.

*3.4. Selected heavy metals in rice*

The concentration of Al, Cu, Fe, Mn, and Zn in the rice samples was determined as shown and illustrated in Table 4 and Figure 4, respectively. The highest amount of Al was determined in long grain parboiled rice from Thailand (64.06 ±0.42 ppm), while the lowest amount of Al was determined in organic white rice from Slovenia (27.95 ±0.06 ppm). The highest amount of Cu was determined in brown rice from the U.S.A. (11.14 ±0.23 ppm), while the lowest amount of Cu was determined in organic white rice from Slovenia (1.36 ±0.00 ppm). The amount of Fe varied between the samples, jasmine rice contained 95.25 ±0.20 ppm of Fe (highest), while organic white rice contained 13.57 ±0.13 ppm of Fe (lowest). The amount of Mn in rice samples ranged between 3.47 to 28.48 ppm, brown rice from the U.S.A. had the highest amount of Mn, while long grain parboiled rice from Thailand had the lowest Mn content. The amount of Zn in the samples ranged between 6.99 to 31.66 ppm, with brown rice from the U.S.A. as the highest sample and organic white rice from Slovenia as the sample with lowest Zn content. Overall, brown rice from the U.S.A. was very rich in terms of Al, Cu, Fe, Mn, and Zn content. Therefore, Fabjola *et al.*, 2015 **[19],** worked on rice husk and rice samples indicated that the concentration heavy metals detected in rice samples decreases in the following order: Mn > Zn > Cu > Ni > Pb > Cr. The metal content in rice husk was higher than in rice.

**Table 4.** Concentration of selected heavy metals in rice samples. The values are reported as mean of 3 replicates ± standard deviation.

| **Rice sample** | **Concentration of heavy metals (ppm)** | | | | |
|---|---|---|---|---|---|
| | **Al** | **Cu** | **Fe** | **Mn** | **Zn** |
| Thai glutinous rice | 38.38 ±0.11 | 2.68 ±0.06 | 15.51 ±0.10 | 8.27 ±0.09 | 14.00 ±0.30 |
| Long grain parboiled rice | 64.06 ±0.42 | 2.76 ±0.02 | 48.65 ±0.27 | 3.47 ±0.01 | 6.99 ±0.14 |
| Basmati rice | 24.92 ±0.25 | 2.55 ±0.05 | 14.28 ±0.16 | 5.20 ±0.00 | 13.17 ±0.11 |
| Punjabi basmati rice | 35.30 ±0.31 | 3.35 ±0.10 | 13.97 ±0.04 | 8.32 ±0.12 | 13.29 ±0.28 |
| Brown rice | 59.97 ±0.25 | 11.14 ±0.23 | 40.58 ±0.22 | 28.48 ±0.24 | 31.66 ±0.26 |
| White pudding rice | 45.22 ±0.22 | 3.28 ±0.12 | 23.18 ±0.18 | 8.47 ±0.13 | 15.05 ±0.13 |
| Jasmine rice | 31.26 ±0.15 | 4.03 ±0.06 | 95.25 ±0.20 | 6.57 ±0.12 | 12.59 ±0.21 |
| Organic white rice | 27.95 ±0.06 | 1.36 ±0.00 | 13.57 ±0.13 | 5.88 ±0.08 | 10.90 ±0.18 |

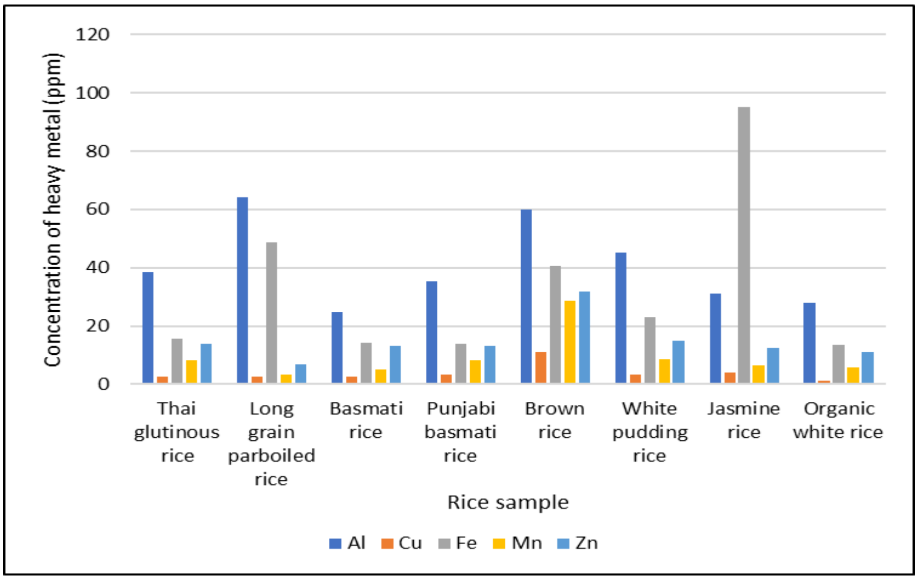


**Figure 4**. Concentration of selected heavy metals in rice samples.

*3.5. Radioactive contamination*

The rice samples were investigated for radioactive contamination by measuring gamma radiation activity concentration. The results are shown and illustrated in Table 5 and Figure 5, respectively. Organic white rice from Slovenia showed the lowest activity concentration (0.02 ±0.00 Bq/kg) which can be considered the safest type of rice in terms of radioactive contamination, this was followed by white pudding rice from U.K., long grain parboiled rice from Thailand, brown rice from the U.S.A., jasmine rice from Italy, Punjabi basmati rice from Pakistan, Thai glutinous rice from Thailand, and basmati rice from Pakistan which showed the highest activity concentration (0.71 ±0.04 Bq/kg). Thus, a study done by Laith, Nada and Fouzey 2015 [20], on long- lived gamma emitting radionuclides in rice consumed in Nineveh Province (Iraq) were performed. The study targeted the natural radionuclides $^{226}$Ra, $^{232}$Th and $^{40}$K. NaI(Tl) detector was used to measure the radionuclides level. The radioactivity concentrations of $^{226}Ra$, $^{232}Th$ and $^{40}K$ ranged from 51.15 to 109.26 Bq/kg,13.67 to 71.97 Bq/kg and 231.87 to 691.71Bq/kg. It was concluded that the rice consumption in Nineveh province (Iraq) is radiologically safe for the presence of the investigated radionuclides.

**Table 5**. Gamma radiation activity concentration of rice samples. The values are reported as mean of 3 replicates ± standard deviation.

| **Rice sample** | **Country of import** | **Activity concentration (Bq/kg)** |
|---|---|---|
| Thai glutinous rice | Thailand | 0.66 ±0.05 |
| Long grain parboiled rice | Thailand | 0.38 ±0.02 |
| Basmati rice | Pakistan | 0.71 ±0.04 |
| Punjabi basmati rice | Pakistan | 0.62 ±0.02 |
| Brown rice | U.S.A. | 0.57 ±0.01 |
| White pudding rice | U.K. | 0.21 ±0.01 |
| Jasmine rice | Italy | 0.61 ±0.03 |
| Organic white rice | Slovenia | 0.02 ±0.00 |

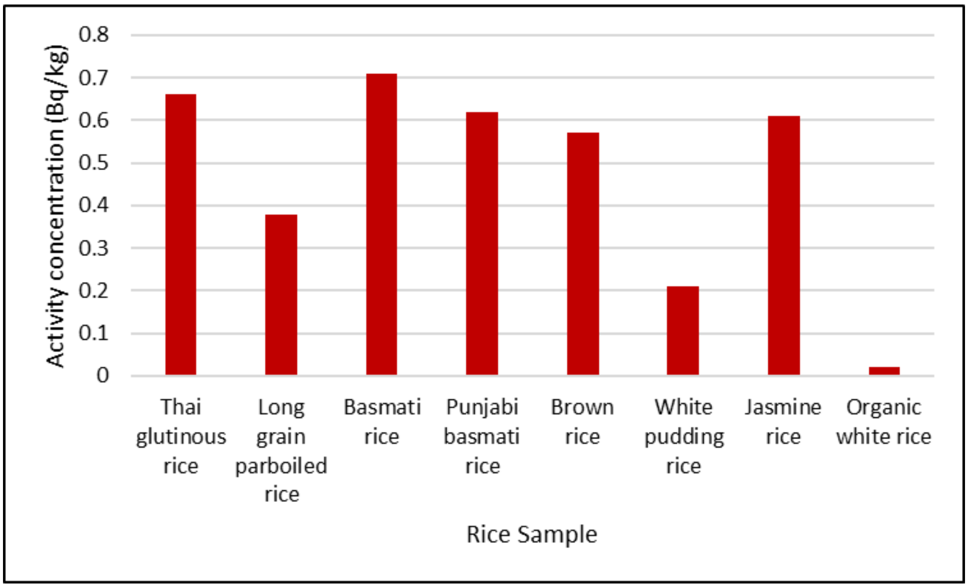


**Figure 5.** Gamma radiation activity concentration of rice samples.

## 4. Conclusions

The protein content, carbohydrate content, inorganic phosphate content, and Al, Cu, Fe, Mn, and Zn content of 8 rice samples imported in Bahrain were analysed. In addition, the gamma radiation activity concatenation of each rice sample was determined. The most significant outcomes of this research were that the highest protein content and inorganic phosphate content was determined in Basmati rice from Pakistan. The highest carbohydrate content and Al content was determined in long grain parboiled rice from Thailand. Brown rice from the U.S.A. had the highest Cu, Mn, and Zn content, while the highest Fe content was determined in Jasmine rice from Italy. The lowest radioactive contaminated rice was the organic white rice from Slovenia.

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