The Estimation of Iron, Zinc, Phytic Acid Contents and their Molar Ratios in Different Types of Bread and Rice Consumed in Halabja City, Iraqi Kurdistan

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Abstract

A total of 10 samples involving five bread and five rice types and five cooked rice were selected. The iron and Zn contents were analyzed using an Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS), and phytic acid content was analyzed using a rapid colorimetric method using a spectrophotometer. Phytic acid to Fe and Zn molar ratios were also determined using their molar weight to determine the potential bioavailability of selected nutrients. A one-way ANOVA test was used to statistically analyze the means differences between the phytate and mineral contents between the bread and rice samples. The variation of Fe and Zn (mg kg\textsuperscript{-1}) in the bread samples was 24.1-65.6 and 13.3-22.8, in row rice was 10.8 to 45.3 and 8.66-17.4, and in cooked rice was 10.0-45.2 and 6.03-15.5 respectively. The PA concentration in (g 100g\textsuperscript{-1}) was 0.77-1.14 for bread, 0.61-1.10 for row rice, and 0.31-0.77 for cooked rice. And also, PA/Fe and PA/Zn ratios were 2.58-6.01 and 8.07-13.4 for bread, 0.25-1.35, and 39.7-110 for row rice, and 0.11-0.89 and 23.0-125 for cooked rice, respectively. The results indicated that soaking and cooking processes caused the reduction in the Fe, Zn, and PA concentrations by 22.4, 5.5, and 27.7 %, respectively. These results from PA/Fe and PA/Zn molar ratios show that Fe had moderate bioavailability while Zn had low bioavailability. The results may be valuable for generate cultivars of wheat and rice varieties with suitable concentrations of PA and micronutrients, which can lead to the improvement of micronutrient-rich cultivars to reduce malnutrition.

Keywords: Bread, Rice, Iron, Zinc, Phytic acid, Molar ratio, Bioavailability.

1. Introduction

Iron (Fe) and zinc (Zn) are the two major important nutritional and health factors to humans. They are vital nutrients and are essential for tissue growth and preservation. The deficiency of these two nutrients is widespread in humans, affecting about 50% of the world population [1]. Furthermore, about 33% of the world population have Fe deficiency and 25% are at risk of chronic Zn deficiency [2,3]. Inadequate intake of these two nutrients causes various problems in humans. Insufficient Fe intake causes the inability to preserve body temperature, rises death of pregnant women and infants, decreases workability and health, and rises exposure to infectious illnesses [3]. Zinc is required for various physiological functions and biochemical processes. It plays an essential role in the metabolism of nucleic acid, protein, carbohydrate and lipids [4]. They reported that low amount of Zn in the human body causes a sequence of metabolic illnesses and even death, their deficiencies are mainly due to inadequate intake of these nutrients from foods such as cereals.

Cereals such as wheat bread and rice are essential dietary sources of various nutrients [2]. These cereals are the staple food for more than 50% of the world population, but they have low bioavailable Fe and Zn to convene human nutrient requirements [5]. Due to the presence of some antinutrients such as phytic acid, could decrease Fe and Zn bioavailability by chelating these nutrients to form inedible complexes in the human body [4]. Bioavailability is described as the portion or amount of the suggested compound that is released from the food substance throughout the intestinal digestion and became available for being absorbed by the intestine [6]. Consequently, improving Fe and Zn content in bread and rice with reducing phytic acid might be an important way to increase Fe and Zn bioavailability. Phytic acid (PA) contains an inositol hexaphosphate-substituted ring complex in its deprotonated form and has high attraction regarding divalent metals in cereal grains and is the main preventive factor in mineral bioavailability, including Fe and Zn [5,7]. However, several processes cause degrading in the PA level including roller milling [1], germination and fermentation [8], and soaking and heat treatment [9]. By these processes can be improved Fe and Zn bioavailability through the reduction of PA levels in cereals and through hydrolyzing by phytase enzyme. It is assumed that, it is vital to have an evaluated food that offers all the...
nutrients required by a human. But we need to believe that part of what we eat is not available. Thus, investing in plans that raise the bioavailability of these micronutrients can be a beneficial choice of progress. Therefore, recovering both Fe and Zn concentration and their bioavailability in bread and rice grains is assumed to decrease the deficiencies of these nutrients to people mainly dependent on bread and rice-based food. There is a lack of information about the bioavailability of Fe and Zn and PA content in staple foods in Iraqi Kurdistan. Thus, the main objective of this study was to evaluate the concentration of the studied micronutrients: Fe and Zn in white bread and rice. Another aim of this study was to determine the PA level and its ratio to Fe and Zn in these two common staple foods.

2. Methods and Materials

2.1 Sampling

A total of 10 different bread and rice samples were selected consisting of five bread types and five different popular types of row rice by convenience sampling. Each raw sample was purchased from three different bakeries and supermarkets in the local market in Halabja city. Each type of sample was then homogenized, powdered using stainless steel electrical grinder and kept at 20°C until further use.

2.2 Rice soaking and cooking

Approximately 500 g of white rice was soaked in 500 mL warm water (40 ºC) (The optimum water ratio in cooking white rice is 1:1) and then the water was removed to prepare the rice to cook. Traditionally, in aluminium bowl (capacity 3L), 50 mL oil and 9 g of salt are heated for about 10 min and then 500 mL of tap water was added and left to boil. After boiling the water, the soaked rice (500 g) was added, mixed all together and then left until the water evaporated for about 30 min and the optimum temperature ranged between 70-85ºC. This is to improve water absorption and accelerate heat transmission which gives ideal texture to all parts of rice. In this study, boiled was reflected adequately when water was absorbed. The time of boiling is measured as the time when rice is heated on the cooker until water is absorbed completely. After that, the cooked rice was cooled at room temperature. Subsamples were dried at 40 ºC for 24h in an oven and powdered, stored in polyethylene tube after labelling for further analysis.

2.3 Elements analysis

The mineral elements were determined in samples with the procedure reported by [10]: the nitric-perchloric digestion. The samples were placed in tubs for absorbance reading with An Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS) (Thermo Fisher Scientific iCAPQ, Bremen, Germany) at the Laboratory of Environmental Analyses from the division of agriculture and environmental science of the University of Nottingham, United Kingdom. The analysis was conducted in triplicate and the results were converted to mg Kg⁻¹.

2.4 Determination of phytic acid

The PA in bread, raw and cooked rice was determined using the procedure described by [11]. Phytic acid was extracted from approximately 0.5 g of sample with using 0.2 M HCl for 3 h followed by centrifugation at 503 g for 30 min. 0.5 mL of extract was mixed with 1 mL of ferric ammonium sulphate solution (0.2 g of NH₄Fe(SO₄)₂⋅12H₂O in 100 mL 2 M HCl and completed up to 1000 mL), incubated in a boiling water bath for 30 min and then cooled in an ice-water bath until reached room temperature. After the cooling, 2 mL of 2,2-bipyridine solution (1% w/v, in distilled water) was added to the mixture. The absorbance was measured immediately at 519 nm and PA concentration in samples was calculated using the calibration curve. Calibration was set up with the respect solutions made by diluting the stock solution (1.3 mg mL⁻¹ PA) with 0.2 M HCl in the range of 0.1 to 1.0 mg mL⁻¹.

2.5 Determination of the molar ratio of phytic acid to minerals

The mole of PA to Fe and Zn was determined by dividing the weight of PA and minerals with its atomic weight (phytate: 660.1g/mol; Fe: 56g/mol; Zn: 65.4g/mol). The molar ratio between PA and mineral was obtained after dividing the mole of PA with the mole of Fe and Zn.

2.6 Statistical analysis

The statistics software Statistical Package for Social Sciences (SPSS) version 26 for Windows was used to analyze the PA, Fe, Zn and PA to Fe and Zn molar ratios and the results were conveyed as average ± standard deviation (SD). The
A comparison of the difference in nutrients, PA and PA to Fe and Zn molar ratios mineral between bread and rice types was analyzed using one-way analysis of variance (ANOVA) analysis. A significant difference was determined by p< 0.05 using triplicate samples from each bread and rice sample were analyzed.

3. Results and Discussions

3.1 Iron and zinc concentrations in bread and rice

We considered the levels of Fe and Zn concentrations for the 10 samples of different types of bread collected from bakeries of Halabja city. In this study, the average values of Fe for the different types of bread were more than Zn. The levels of Fe and Zn in the samples ranged from 24.1±12.6 to 65.6±10.2 and from 13.3±0.60 to 22.8±0.50 mg kg\(^{-1}\) respectively and the mean contents of these nutrients were 65.5 and 22.5, 24.1 and 17.0, 38.5 and 22.5, 24.9 and 13.3, and 45.7 and 22.8 mg kg\(^{-1}\) for Lawasha, Mashini, Tiry, Samoon, and Hawrami respectively (Fig.1). The levels of Fe and Zn had a significant difference between different types of bread (p≤0.05). The variation of Fe and Zn concentrations between bread types were 41.5 and 9.55 mg kg\(^{-1}\) respectively. The level of Fe in bread samples showed a large variation. The high concentration of Fe in Lawasha bread was approximately three times higher than the lowest concentration in Mashini bread and the high concentration of Zn in Hawrami bread was approximately two-times higher than the lowest concentration in Samoon bread.

We found the lowest Fe concentration in Mashini bread and the highest in Lawasha bread, whereas the lowest Zn concentration was found in Samoon bread and the highest in Hawrami bread. Rasheed and Hama Salih [10] reported the content of Fe in the same bread types to be 13.2±3.18-26.2±8.24 mg kg\(^{-1}\). Harmankaya, Ozcan [12] found that the content of Zn in the bread was 20.9 mg kg\(^{-1}\). Khokhar, King [13] reported Zn concentration in the white bread was 8-15 mg kg\(^{-1}\).

The results indicate that the concentration of Fe in bread samples is higher than the permissible level of 5.0 mg kg\(^{-1}\) reported by [14], which is showing that the bread consumed in Iraqi Kurdistan is a good source for Fe daily intake. Moreover, the results in the present study showed that the Fe concentration is higher than the same type of bread reported by [10]. They studied the concentration of Fe in different bread types consumed in Sulaymaniyah province. Ting and Loh [15] found Fe and Zn concentrations were 52.8 and 14.7 in white bread in Malaysia. The difference may be due to environmental conditions such as rain, the weather during cultivation of wheat grain, and application of chemical fertilizer which is reported by [16].

The high Fe concentration in the studied bread types may also be due to equipment used during the bread baking process which is well recognized that is a source of increasing metals such as Fe and Zn. Rasheed and Hama Salih [10] reported an increase in Fe concentration (14.5-44.6%) during bread making process for the same types of bread. Another reason for increasing Fe concentration may be returned to the use of water which contains some heavy metals. By this, the concentration of metals will increase in the final product.

We also measured the levels of Fe and Zn concentrations for the five types of rice samples collected from local markets in Halabja city. In this study, the average Fe concentration for the different rice types was more than Zn. The concentrations of Fe and Zn in the rice samples ranged from 10.81±3.6 to 45.3±2.91 and from 8.66±0.60 to 17.4±10 mg kg\(^{-1}\) for Mahmood and Saman row rice types respectively. (Fig.2). A significant difference was found between rice types under (p≤0.05). After cooking the percentage of Fe and Zn reduction were ranged between 6.05-26.2 and 11.3-39.0% respectively. the reduction of...
Fe and Zn concentrations may be due to removing some of these two nutrients during the soaking process. People when they cook rice start with soaking the rice for about 1h in hot water. This is cause removing most of the nutrients in the outlier of the grain so which causes a decrease in their concentrations. High variation of Fe and Zn concentrations between rice types were found may be due to the genotype, cultivation condition and fertilizer application. Norhaizan and Nor [17] reported Fe and Zn concentrations for different Malaysian rice brands were 4.7-70 and 1.4-16.4 mg kg\(^{-1}\) respectively. Kumar, et al. [18] reported the highest concentration of Fe and Zn of 24.15 and 35.5 mg kg\(^{-1}\), respectively in Indian rice. Compared to those reported, the concentration of Fe and Zn in rice consumed in Iraqi Kurdistan is in the range reported globally. However, plant breeding programs in bio fortification of staple food crops need to study rice varieties, and select types having high Fe and Zn concentrations to be used as contributor parents [19]. An increase in the concentration of Fe and Zn in grain is a high-significance research area. The development of a great genetic variant for Fe and Zn present in cereal germplasm is an important method to reduce the amount of Fe and Zn insufficiencies in the developing world [18]. In this study, five local rice types were analyzed for Fe and Zn content among the rice grain for Fe and Zn concentration, the Saman type was found to be high for both Fe and Zn but Mahmood was found to be low Fe and Zn before and after cooking. 

![Figure 2](image-url)

**Figure 2.** The concentrations iron and zinc in the five studied raw and corresponded cooked rice types.

### 3.2 Phytic acid concentration and PA/Fe and PA/Zn molar ratio

Mineral bioavailability such as Fe and Zn in food grains is commonly poor due to the presence of some anti-nutritional factors such as PA, tannin and dietary fiber [20]. Improving the bioavailability of these micronutrients is a challenge that is mainly essential for developing countries. This could be reached by a screening of obtainable genotypes with higher concentrations of Fe and Zn in the cooked part and lower the concentration of complexes that prevent their absorption, such as PA [21]. Table (1) summarized the PA concentrations, PA/Fe, and PA/Zn molar ratios of bread and rice samples eaten in Halabja province. In bread, Samoon bread had higher PA concentrations, followed by Lawasha, Hawrami, Tiry, and Mashini, but the PA/Fe and PA/Zn molar ratios in Samoon bread were higher followed by Mashini, Tiry, Hawrami, and Lawasha bread, significant differences (p≤0.05) were found between bread. Phytic acid in the studied bread samples ranged between 0.77 to 1.14 g 100g\(^{-1}\), which is higher than that found in Pakistani (0.75 g 100g\(^{-1}\)) flatbread. Roohani, et al. [22] reported a lower PA concentration for Iranian flat flatbread. They found PA concentration was 0.25-0.49 g 100g\(^{-1}\). However, Qazi et al. [23] found a similar PA concentration in Pakistani bread was 0.66-0.75 mg 100g\(^{-1}\). A high variation of PA in the studied bread samples was observed which is larger than that observed in the Iranian and Pakistani bread. The bread-making processes in the three cultures are similar. Compared to those, the concentration of PA in the studied bread is higher than reported globally. Throughout the transformation of flour into bread, PA content reduces as a result of the activity of natural phytase. Decrease of PA content during the bread-making process depends on phytase action, which in turn is affected by various reasons, such as the level of flour extraction, the yeast, the acidity of the dough, the proofing time and temperature, and enzymes added to the dough and the presence of calcium salts [24, 25].

Furthermore, the PA/Fe and PA/Zn molar ratio ranged between 2.58-6.01 in Lawasha and Samoon bread and 8.07-13.4 in Hawrami and Samoon bread respectively. The increasing PA concentration decreased the bioavailability of Fe and Zn. The reduction of PA/Zn ratio to between 1 to 0.4 and PA/Zn to less than 5 has been estimated as the threshold for improving Fe and Zn bioavailability in cereals [24, 26]. However, in general, the estimated molar ratio of PA/Zn and PA/Zn for bread were higher than 1 and 5 respectively [27] which limits the bioavailability of Fe and Zn. Considering the mean values of PA/Fe and PA/Zn ratios, the studied bread samples revealed low Zn bioavailability according to [21]. And Jambrec, et al. [27] They reported that only 55% of the Zn content of diets is estimated to absorbed if the PA/Zn ratio of diets is less than 5, but only 35% of Zn would be absorbed if the ratio is between (5-15) and less than 15% would be absorbed if the ratio is more than 15. Therefore, Zn bioavailability in bread in Halabja city is low (35%) due to the PA/Zn molar ratio (less than 13.4 in all the
bread samples. Low PA concentration in breads may result from the fermentation process decreases PA content due to the action of endogenous and microbial phytases in flour which together cause hydrolysis of PA [17, 28]. Fron tela, et al. [29] discovered that the baking process decreased PA content from 58.6 to 9.4 mg 100g⁻¹ in white bread. Among the five rice types are commonly eaten in Halabja city were selected to determine PA concentration in a row and cooked rice. The highest PA concentration was found at 1.10 g 100g⁻¹ in Saman type and lowest 0.74 g 100g⁻¹ in Marjan type (Table1). Kumar, et al. [20] reported a higher range of PA (0.82-2.62 g 100g⁻¹) in some row rice genotypes. While Kumar, et al. [18] found a similar PA concentration of 0.912 g 100g⁻¹ in six Indian rice varieties. Compared to those reported, the PA concentration in commonly consumed rice is in the range reported globally. After cooking the percentage of PA concentration reduced was between 16.3 and 72.1% in Marjan and Saman types respectively. The high reduction of PA in the studied rice types after cooking is due to soaking and heating processes which is well documented by [9]. Jing, et al. [30] studied the impact of the heating process on Fe and Zn bioavailability in cereals and reported that the heating process cause decreases the PA concentration in the cereals. Kar kale and Beleia [31] studied the effect of soaking and cooking on PA concentration in cowpea. They observed a reduction in PA concentration during soaking and cooking processes (23-30%). In raw and cooked rice, the highest PA/Fe and PA/Zn ratios were found in Mahmood and the lowest in Grda, even though these ratios changed between raw and cooked. In all rice types, PA/Fe molar ratio decreased after cooking while PA/Zn in all rice types increased except in the Saman type. The decrease in the PA/Fe molar ratio is due to the degradation of PA during rice cooking, but increasing the PA/Zn molar ratio may be due to the removal of some amount of Zn in the rice. This is because normally people remove soaking water before cooking. Suri and Tanumihardjo [32] studied the effects of different methods on micronutrients content in maize and found that soaking and cooking processes cause a decrease in the concentration of micronutrients. Moreover, Jing, et al. [30] studied heat-processing on Fe and Zn bioavailability in rice found that about 58.45 of Zn bioavailability decreased due to heat-processing. They also reported that a decrease in Zn bioavailability on heat treatment of the tested food grains could be attributed to interactions of Zn with proteins, and/or further food constituents thus obstructive its assimilation. Overall, the average PA/Fe and PA/Zn were 0.69 and 73.4 for raw rice and decreased to 0.52 and 70.2 for cooked rice, respectively.

The molar ratios of PA/Fe of all raw and cooked rice types were between 0.2-3 while the molar ratio of PA/Zn was higher than 79. These ratios indicate that all the rice samples had poor bioavailability of Fe and Zn. This could be due to the high content of PA in the studied rice types which impacts the Fe and Zn bioavailability in this food. Another probability could be due to a loss of nutrients during the polishing process, the main process to produce white rice and soaking the rice before cooking. Most of the minerals in rice can be discovered in the rice bran. But, during the polishing process, the rice bran is removed along with its nutrient composition.

The differences between our samples and those from other studies could stem from both the type of samples included in these studies and the methods of analysis used. AAS (atomic absorbance spectrophotometry) was used for Fe and Zn analysis in the Iranian and Pakistani and Chinese samples. Other studies (including this study) used the ICP-MS method for mineral analysis. Procedures of pre-treatment of samples, their digestion, and sensitivity limits of equipment might also affect the absolute levels of minerals reported in rice.

Table 1. The concentration of phytic acid and its molar ratio to iron and zinc in bread and raw and corresponded cooked rice types analyzed.

| Studied samples | Type       | PA g 100g⁻¹ | PA/Fe | PA/Zn |
|-----------------|------------|-------------|-------|-------|
| Bread           | Lawasha    | 0.97±0.23   | 2.58±1.13 | 8.52±3.07 |
|                 | Mashini    | 0.77±0.16   | 5.45±2.19 | 8.27±2.30 |
|                 | Tiry       | 0.88±0.19   | 4.64±3.00 | 10.0±7.13 |
|                 | Samoon     | 1.14±0.11   | 6.01±1.86 | 13.4±5.03 |
|                 | Hawrami    | 0.89±0.12   | 3.88±2.25 | 8.07±2.83 |
|                 | Saman      | 1.10±0.16   | 0.37±0.07 | 73.3±128.5 |
|                 | Grda       | 0.61±0.008  | 0.25±0.02 | 39.7±0.43 |
| Raw rice        | Khiam      | 0.92±0.10   | 1.10±0.02 | 63.9±15.6 |
|                 | Mahmood    | 0.96±0.02   | 1.35±0.10 | 110±67.6 |
|                 | Marjan     | 0.89±0.03   | 0.40±0.05 | 79.7±35.5 |
|                 | Saman      | 0.31±0.05   | 0.11±0.05 | 21.3±12.1 |
|                 | Grda       | 0.53±0.06   | 0.24±0.06 | 42.1±2.64 |
| Cooked rice     | Khiam      | 0.77±0.14   | 0.89±0.14 | 64.4±16.7 |
|                 | Mahmood    | 0.75±0.08   | 0.89±0.08 | 125±97.3 |
|                 | Marjan     | 0.75±0.08   | 0.45±0.06 | 96.2±58.3 |

Values are stated as average ± standard deviation, SD (n=3)
Conclusion

In conclusion, variable concentrations of Fe, Zn, and PA can be found in the different types of bread and rice generally consumed in Halabja City, Iraqi Kurdistan. Difference in Fe, Zn, and PA were not only detected in different types of the studied samples, but also in the same types after manufacture. Phytic acid to Fe molar ratio below 0.2-3.0 for studied samples, indicated moderate to high Fe bioavailability but PA to Zn molar ratio showed that all the studied samples had low Zn bioavailability. These results showing that while the samples had high Fe and Zn contents, they furthermore had a high PA content which may decrease the bioavailability of Fe and Zn to the body. Thus, perfect food processing and cooking techniques should be selected to reduce this effect.

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