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Research article

CONSEQUENCES OF COOKING METHOD IN ESSENTIAL AND HEAVY METAL CONTENTS IN BROWN AND POLISHED ALIKAZEMI RICE

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ABSTRACT: It has been reported that as much as 75% of the daily calorie intake of the people in some Asian countries is derived from rice. At harvest time (August to late September 2013) 800 Alikazemi rice samples were randomly collected and were studied in states of: Whole rice grains, Brown and Polished in 4 different states of heating and cooking processes: Raw , Rinsed, Cooked and Drained. Samples collected from ten major farmland rice production areas in Gilan province in the north of Iran from cities due to investigation the effect of milling, cooking and heating processes in polished and brown rice samples on their mineral and heavy metal contents(Mn, Zn, Fe, Pb, Cd and As) and find the best cooking methods in order to avoid the side effects of heavy metals in edible an consumed rice and also assess the associated health risk posed to the population through exposure to heavy metals (Arsenic, Cadmium and Lead) in rice. Results show that rinsing samples especially after 4 times has the significant role ($p< 0.002$) in decreasing the cadmium and lead contents. The results of this research revealed that the removal of husk layer during dehusking and polishing substantially reduces the mineral nutrient value of rice grains. In general, the bran contains higher levels of iron, Manganese and Zinc. Polishing substantially reduces the mineral nutrient value of rice grains. The results give us the possibility to process brown rice to obtain Low toxic and heavy metals contents at a relatively high zinc, Iron and Manganese content.
Key words: Heavy metal , Brown rice, pollished rice, cooking method, Heating process.

INTRODUCTION

It has been reported that as much as 75% of the daily calorie intake of the people in some Asian countries is derived from rice [1, 2]. The environmental impacts of rice depend on the location of cultivation, variety, processing and forms of rice [3-8]. It is reported that half of the World's households cook daily with biomass energy. Domestic cooking-stoves either traditional or improved used for cooking in rural areas cause incomplete combustion of biomass and emit gaseous substances and suspended particulates which are responsible for many health problems [9, 10]. In Asia, rice is the main dietary source for energy, protein, thiamine, riboflavin, niacin, iron and calcium [11-13]. Rice cultivation in the Northern Province areas of Iran has a long history, especially Somesara and Rudbar**.** Also rice is the second high consumption food among Iranian people [14]. It is the most common crop grown in agricultural lands in the north of Iran [15-17]. Today, the population growth in Iran's domestic production of rice to meet the needs of the people and therefore, a significant amount of this product is imported from abroad despite Iran's rice acreage is only 600 hectares, the amount of land under cultivation is very small world. But there are different varieties and has a central role in discussions of research and research training is remarkable [17]. Rice properties are known to be dependent on the variety of rice, methods of cultivation, processing and cooking conditions. Some factors such as soil, pH and environmental conditions can affect to metal contents in plant products for example any reduction in pH soil in these farms could raise metal availability and metal Uptake by plants , which also could increase health risk. It is also known that there is a linear relationship between metal availability and organic matter content [18].

Rice may contain As in total concentrations up to 100–400 ng/g, including both inorganic As (As_i) and the organic species dimethyl-arsenate (DMA) (Williams et al. 2005), with much lower concentrations (relative to DMA) of mono-methyl-arsenate (MMA) also occasionally detected. Total As (As_{total}) in rice and relative proportions of DMA and As_i differ both geographically (Meharg et al. 2009) and as a function of genetic and environmental controls [19].

Although the parboiling treatment helps in retaining some of the nutrients, reduce breakage loss during milling and increases head rice recovery (whole rice kernels after milling), a considerable amount of energy and labor is consumed [2]. The commercial rice-milling process leads to products with low-value fractions, such as husk and bran. Hand pounding of paddy in a mortar with a pestle is still practiced in some remote areas. The rice grain consists of four major tissues: the hull, embryo, aleuronic layer, and starchy endosperm. The endosperm is the most important grain fraction with respect to human nutrition, as it is the part of the grain primarily consumed in many countries [20]. White rice, the main consumption product, is referred to as polished or whitened rice when 8-10% of mass (mainly bran) of the outer part has been removed from brown rice [12,21]. Rahaman et al. reported that milling should be restricted to 7 to 8% for maximum recovery, which is the common practice in most developing countries, including Bangladesh [22]. The biggest nutritional shortfall of rice as a staple food is its low content of protein, iron, iodine and vitamin A. In the world, 3.5 billion people were reported iron deficient [21]. Milling brings about considerable losses of nutrients and affects the edible properties of milled rice [12, 23, 24]. As most cereals, rice does not show a homogeneous structure from its outer (surface) to inner (central) portions [25]. In addition to the effect of equipment and process conditions, the composition, structure and thickness of rice kernels also affect the extent of breakage from milling [26,27]. On the other hand human faces to heavy metal contamination during recent years. The heavy metals may transfer in the environment, and on the higher steps of the food pyramid, they may result in a sudden intoxication or ailments [28]. The danger created by the accumulation of these heavy metals at the higher level of the food chain is regarded as one of the main concerns in the human health [29]. The available heavy metals in the environment are regarded as one of the potential dangers for the organisms. Human as well as the animals are always faced with the pollution with heavy metals [30]. As other recent researches have showed some heavy metals contamination in crops and specially rice varieties product therefore this research builds upon:

- Determination and comparing heavy metals (Lead, Cadmium and Arsenic) contents in Ali Kazemi raw rice variety samples in parts of rice grains after separation of whole grains into hulls and brown rice, and then milling the brown rice into bran and polished rice samples collected from ten major rice paddy (farmlands) from ten cities in Gilan Province: Rasht, Shaft, Khomam, Masal, Soumahe Sara, Roudbar, Fuman, Astaneh-e Ashrafiyyeh, Lashtenesha, Kochesfehan in the north of Iran.
- Investigation the effect of milling, cooking and heating processes in polished and brown rice samples on their mineral and heavy metal contents(Mn, Zn,Fe, Pb,Cd and As).
- Apply the best cooking methods in order to avoid the side effects of heavy metals in edible an consumed rice.
- Assess the associated health risk posed to the population through exposure to heavy metals (Arsenic, Cadmium and Lead) in rice.

MATERIAL AND METHODS

Study Area

Gilan Province is one of the 31 provinces of Iran. It lies along the Caspian Sea, just west of the province of Mazandaran, east of the province of Ardabil, north of the provinces of Zanjan and Qazvin. The northern part of the province is part of territory of South (Iranian) Talesh. At the center of the province is the main city of Rasht. Other towns in the province include Khomam, Lashtenesha, Astaneh-e Ashrafiyyeh, Fuman, Lahijan, Langrud, Masooleh, Masal, Rudbar, Roudsar, Shaft, Talesh, and Soumahe Sara. The name and description of all the sites in this study are provided in figure 1.

Sampling method

At harvest time (August to late September 2013) 800 Alikazemi rice samples were randomly collected and were studied in states of: Whole rice grains, Brown and Polished in 4 different states of heating and cooking processes: Raw , Rinsed, Cooked and Drained samples collected from ten major farmland rice production areas in Gilan province in the north of Iran from cities : Rasht, Shaft, Khomam, Masal, Soumahe Sara, Roudbar, Fuman, Astaneh-e Ashrafiyyeh Lashtenesha, Kochesfehan and each samples consists of 20 subsamples . All sample sites were recorded using a hand-held Global Position System (GPS).As the aim of this study was determination of mineral and heavy metals : Fe, Zn, Mn, Cd, Pb and As in 4 different states : whole raw rice grains, brown and polished rice(raw, rinsed, cooked and drained states). All samples collected at the same time from each paddy.

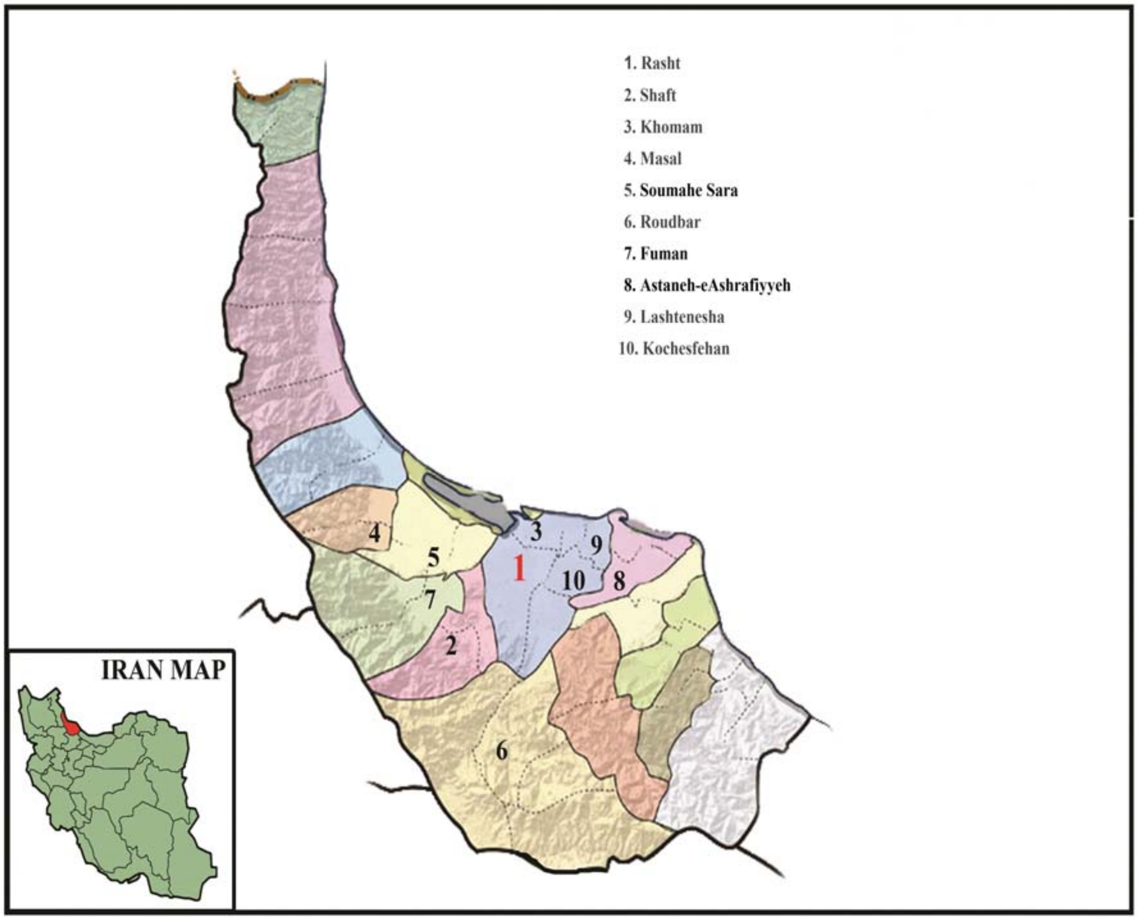

Figure 1- Description of rice sampling

Preparing method

Lead and cadmium Determination

All glassware and plastic containers used were washed with liquid soap, rinsed with water, digested in 10% v/v of nitric acid for 24hrs, cleaned thoroughly with distilled water and dried in such a manner to ensure that any contamination does not occur. For heavy metal analyses 50 gram of each sample was weighed and oven-dried at 60^0c to a constant weight.

Each oven-dried sample was ground in a mortar and passed through a 0.25 mm pore size 60 mesh sieve. A digestion mixture comprising of concentrated HNO_3 (69% Merck and hydrochloric acid 37% Merck in the ratio of 3:1 v/v was used for wet digestion of the samples. Ten grams of powdered and oven-dried sample was weighed precisely on electronic balance (Shimadzu LIBROR AEX 200G). The samples were put in a 100 ml digestion flask and 20 ml of digestion mixture was added to it and heated on a hot plate in the fuming chamber. Blanks (10% v/v of nitric acid) and samples were also processed and analyzed simultaneously. All the chemicals used were of analytical grade (AR). This method has been followed in 4 stages for raw rice (untreated samples, rinsing rice, draining and cooked rice. The rinsing samples prepared by washing 4 times and in each step proportion of water and rice was 4:1 and for preparing of cooking rice bring the water to boil and then add 10 gram of oven-dried samples when the water has come to boil(rice is cooked in lots of water just like pasta) for 10 minutes then drain it in a colander and wash it by cold water just one time (traditional method for preparing rice in Iran) [17] and for preparing draining rice samples put the oven-dried samples into boiling water and then heating the plate for 10 minutes till the steam escape . All draining and cooking rice samples have rinsed 4 times then followed by the procedure.

Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents by wet digestion method and atomic absorption spectrophotometry analysis based on annual book of ASTM standards and AOAC [31-33]. All digested sample flasks were firstly heated slowly and then vigorously till a white residue is obtained. The residue was dissolved and made up to 10 ml with 0.1 N HNO_3 in a volumetric flask.

The samples were analyzed by a Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame for heavy metals: lead and cadmium, using at least four standard solutions for each metal [32]. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines [31].

Arsenic Determination

Perkin Elmer Single-Element Calibration Standards for Atomic Spectroscopy were used as the stock standards for preparing the working standards (Part Nos. As: N9300180). Working standards were prepared by serial volume/volume (v/v) dilution in polypropylene vials [34-35]. All working standard solutions were freshly prepared the day of analysis from the stock standards [34]. The samples and standards for arsenic analysis were pre-reduced (As^{+5} to As^{+3}) prior to analysis. This was achieved by adding a reducing solution containing 5% (w/v) KI and 5% (w/v) ascorbic acid. An appropriate volume of standard or sample (up to 10 mL) was placed in a 50 mL polypropylene auto sampler tube. To this, 1 mL of the reducing solution and 5 mL of concentrated HCl was added. The treated samples or standards were set to stand at room temperature for 30-60 minutes prior to analysis. The tube was brought to the 50 mL mark with deionized water and the sample was ready to run. The final dilution factor for the SRMs was 10 [34].

Zinc, Manganese, Copper Determination

For Zinc, Manganese, Copper and Selenium concentration 50 gram of each prepared rice sample was weighed and oven-dried at $80C^0$ to a constant weight. Each oven-dried sample was ground in a mortar until it could pass through a 60 mesh sieve. The samples were stored in clean, dry, high density polyethylene bottles of 100 ml capacity with screw caps. Finally 5 gram of dried sample was weighed precisely on electronic balance (Shimadzu LIBROR AEX 200G). The samples were put in a 100 ml digestion flask and 20 ml of digestion mixture comprising of concentrated HNO_3 (65%) Merck and hydrochloric acid (70 %) Merck in the ratio of 3:1 was added to it and heated on a hot plate in the fuming chamber. Blanks and samples were also processed and analyzed simultaneously. All the chemicals used were of analytical grade (AR). This method has been followed in 5 stages: whole rice grains, hulls, brown rice, bran and polished rice. Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents [31, 33]. The flasks were firstly heated slowly and then vigorously till a white residue is obtained. The residue was dissolved and made up to 10 ml with 0.1 N HNO_3 in a volumetric flask. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame, using at least five standard solutions for each metal.

Iron Determination

The aliquot was passed through the atomic absorption spectrophotometer to read the iron concentration. Standards were prepared with a standard stock of 10 mg/L using ferrous ammonium sulphate where 3 - 60 ml of iron standard solution (10 Mg /L) were placed in stepwise volumes in 100 ml volumetric flasks. 2 ml of hydrochloric acid were added and then brought to the volume with distilled water. The concentration of iron in the aliquot was measured using the atomic absorption spectrophotometer in mg/L. The whole procedure was replicated three times [33].

Statistical Method

State differences on the basis of the states (Brown rice and polished rice) of samples and different sates of cooking (Raw, Rinsed, Drained and cooked) were determined by student t-test. The changes were calculated by one way ANOVA and for analysis of the role of multiple factors univariate analysis was used by SPSS 17.Probability values of <0.05 were considered significant. Concentrations were expressed in terms of mg/Kg on a dry weight basis.

RESULTS AND DISCUSSION

The results of Cadmium and Lead contents in 800 samples of raw, rinsing , boiling – drained and cooking brown and polished Alikazemi rice from 10 main agricultural areas in Gillan province; the north of Iran are shown in figure 2. All concentrations are expressed as mg /kg DW. Results show that the mean content of Cadmium and Lead in the most samples from Gillan province is over. ANOV A analysis showed that there was a significant difference in Pb contents in brown and polished rice samples ($p < 0.05$) . The minimum and maximum Pb content in rinsing brown rice and cooked polished rice was 1.0112 and 2.5418 mg/kg DW. The results in figure 2 revealed that all brown and polished rice samples (100%) had lead content above maximum level 0.3 mg/kg and Cadmium content in all samples except rinsing and raw brown rice were above maximum level 0.2 mg/kg which is recommended by FAO/WHO Expert Committee on Food Additives [36,37,38].

Cooked polished rice had the highest level of lead while drained polished samples had the highest level of Cadmium which means the highest level of heavy metals were seen in polished samples. The minimum and maximum Cadmium content respectively belongs to rinsing brown rice by 0.1002 mg/kg DW and drained polished rice 0.4432 mg/kg DW.

Rinsing samples especially after 4 times has the significant role ($p < 0.002$) in decreasing the cadmium and lead contents. For inter-area differences in Manganese and Zinc elements in the rice while the statistical analysis shown no significant differences (p 0.05) among the different farmlands in 10 different cities in Gilan areas although in Astaneh-e Ashrafiyyeh Lashtenesha, Kochesfehan the iron content was meaningfully more than other studied areas (p 0.03) which probably related to characteristics of the soils of these geographical areas.

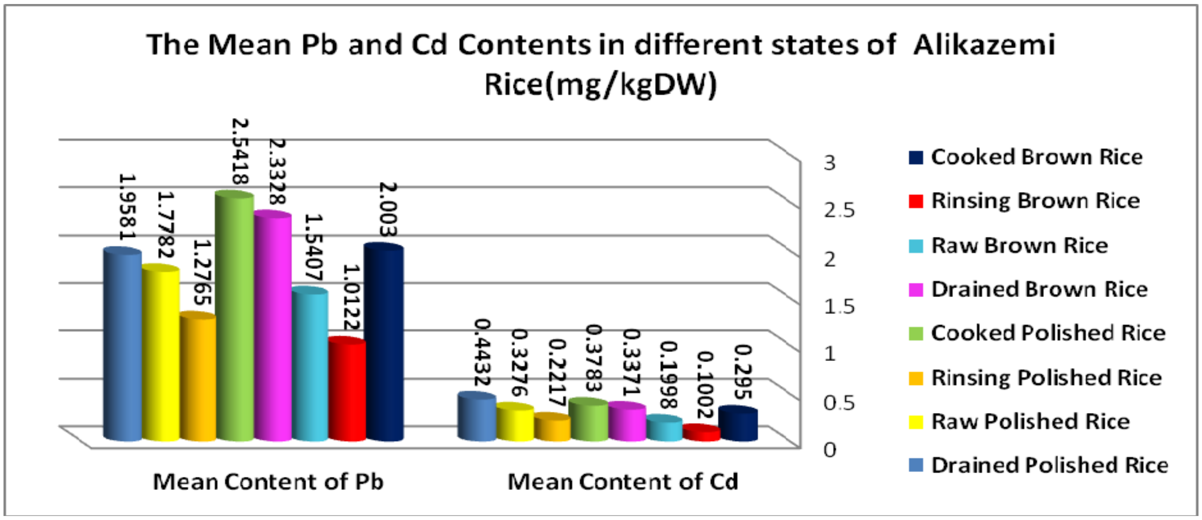

Figure 2-Comparison the mean level of Lead and Cadmium contents (mg/kg DW) in different states of cooking Alikazemi rice

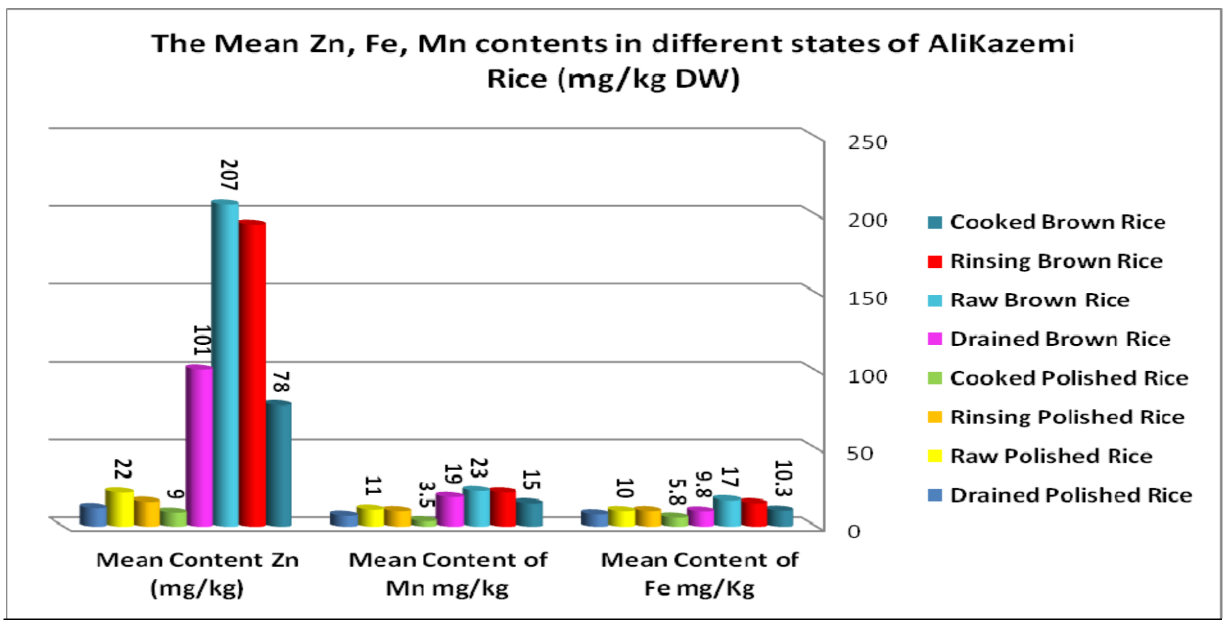

Figure 3-Comparison the mean level of Zinc, Iron and Manganese contents (mg/kg DW) in different states of cooking Alikazemi rice.

The Zn concentration of raw brown rice was about 9.4 times the concentrations in the raw polished rice and about 2.6 times greater than cooked brown samples. Although cooking and heating reduce the zinc content but the mean zinc content in cooked brown rice had about 2 times than drained brown samples. The concentration of Fe in raw brown samples was 1.7 times greater than in raw polished samples and cooked brown samples in comparison by cooked polished samples had 2 times more iron.

Variation in the other elemental concentration manganese showed same trend and the Mn concentration in the raw brown samples was 2.1 times its concentration in raw polished rice. Concentrations of Mn in drained brown were 3 times higher than those in the polished rice. In polished rice the concentration of all studied mineral elements was much lower than brown forms. The same study has performed for Arsenic concentration. As it has been shown in figure 4 only in raw and cooked polished rice this toxic metal was detectable which means likely mechanical mechanism can lead to release arsenic from complex for to free ion form as same as other studies [39].

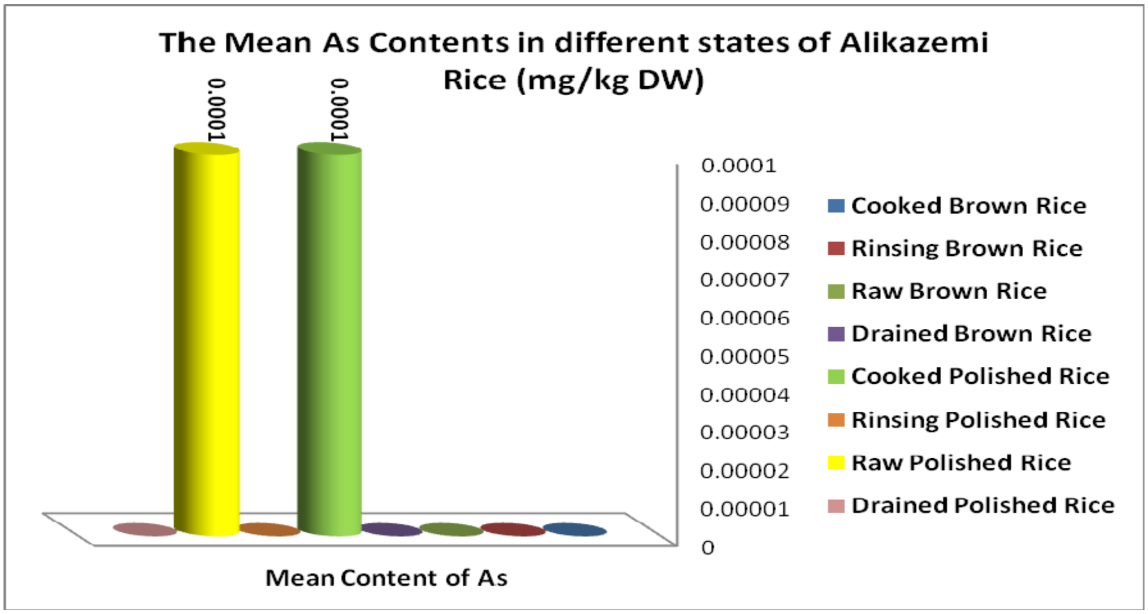

Figure 4- The mean arsenic content in different states of Alikazemi rice (mg/kg DW)

CONCLUSION & RECOMMENDATION

The present study examined the baseline of essential and trace elements in 800 rice grain samples produced in ten administrative areas of North province of Iran in 2013. One of the most interesting findings of this research is that Rinsing process especially after 4 times has the significant role ($p< 0.002$) in decreasing the cadmium and lead contents. Due to Lead and Cadmium contamination this result is very important for consumer in order to prevent heavy metal toxicity. The results of this research revealed that the removal of husk layer during dehusking and polishing substantially reduces the mineral nutrient value of rice grains. In general, the bran contains higher levels of iron, Manganese and Zinc. Polishing substantially reduces the mineral nutrient value of rice grains. From this study we conclude that milling characteristics, including mass loss and breakage, varied with rice kernel shape and geographical and physicochemical properties of rice samples. Most of Zinc and Iron and Manganese contents in rice kernels is located in the outermost layer. The results give us the possibility to process brown rice to obtain Low toxic and heavy metals contents at a relatively high zinc, Iron and Manganese content.

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