

ORIGINAL ARTICLE

# Effects of Two Cooking Methods on the Concentrations of Some Heavy Metals (Cadmium, Lead, Chromium, Nickel and Cobalt) in Some Rice Brands Available in Iranian Market

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## KEYWORDS

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**ABSTRACT:** Dietary intake is the most important route of exposure to heavy metals for most people. Rice is a staple food for Iranians that, if polluted, threatens public health. Cooking process may affect heavy metals content of rice. In this study, concentrations of cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni) and cobalt (Co) were measured in 3 brands of imported rice available in Shiraz-Iran market. Two methods of cooking (Kateh and Pilaw) were applied for all brands. The heavy metals were detected in raw and cooked rice grains. The samples were analyzed by atomic absorption spectrometer. The mean concentrations of Cd, Pb, Cr, Ni and Co were 0.33, 1.75, 0.38, 0.89 and 0.20 µg/g in raw rice samples respectively, 0.10, 1, 0.29, 0.19, and 0.03 µg/g in Kateh rice samples and 0.10, 0.98, 0.28, 0.18 and 0.03 µg/g in Pilaw rice samples. Cooking rice grains reduced the content of heavy metals. Minimum cooking efficiency was for Cr. The effect of cooking methods was not significantly different in this study. The obtained concentrations were compared with the permitted values, published by Institute of Standards of Iran, for heavy metal contents in rice. Moreover, weekly intakes of the metals through rice were estimated and compared with the PTWI (Provisional Tolerable Weekly Intake) amounts determined by FAO/WHO.

## INTRODUCTION

Heavy metals are one of the major contaminating elements in our food supply [1]. Serious health hazards

can be created as a result of extreme dietary accumulation of heavy metals such as Cr, Cd, Ni and Pb [2]. It is believed that Lead and cadmium are especially

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toxic [3]. Cd exposure may cause kidney damage and also bone effects and fractures [4, 5]. Furthermore, long-term exposure to Pb is known to pose health risks such as memory deterioration, prolonged reaction times and reduced ability to understand [4]. Although inhalation is a main factor for heavy metal conduction in very contaminated sites, dietary intake is the most important route of exposure to heavy metals for most people [6]. Thus, the detection of heavy metal concentrations in food products and their dietary intake is very important for assessing their risk to public health [7]. The main sources of heavy metal contamination in agricultural crops are irrigation with contaminated water, fertilizers and metal based pesticides, industrial emissions, transportation, harvesting process, storage and/or sale of crops [8, 9, 10]. Rice is one of the major agricultural products on the international market [11]. Many researchers have reported heavy metal concentrations, especially for Cd, Pb, Cr and the other elements in rice grains from various countries [12, 13, 14, 15 and 16]. In this study rice was adverted for its individual consumption as a staple food in Iran [17] and probable hazards of its heavy metal contents on population health. Rice variety, treatment of rice and diversity of cooking may affect elemental content and intake of heavy metals [11]. The objective of this study was to determine Cd, Pb, Cr, Ni and Co contents of some imported raw and cooked polished rice in Shiraz market and the effects of different ways of cooking on the content of their heavy metals.

## MATERIALES AND METHODS

### *Material*

All Chemicals, reagents and standards for heavy metals with maximum purity were obtained from Merck, Germany. High purity de-ionized water used in sample and solution preparation was obtained using a Milli-Q water purification system (Millipore RiOs-DITM, Bedford, MA, USA). A clean laboratory and laminar-

flow hood capable of producing class 2 were used for preparing solutions. Plastic bottles and vessels were cleaned by soaking in 8% (v/v) HNO<sub>3</sub> for 48 h, rinsed three times with de-ionized water and dried in a class-2 laminar flow hood before use. All operations were performed in, Central Laboratory of desert region management department of Shiraz University.

### *Apparatus*

This study was conducted in order to evaluate some heavy metals such as cadmium, lead, chromium, nickel and cobalt. The instruments used in this research include a Milli-Q water purification system (Millipore RiOs-DITM, Bedford, MA, USA), Atomic absorption spectrometer (Shimadzu AA-680 and Shimadzu MUV-IA, Tokyo, Japan).

### *Sampling*

3 brands of imported polished rice grains are named Abdossaeid, Maryam and Tajmahal were collected from market places in Shiraz, Iran. Rice is imported to Iran market from Pakistan, India and Thailand. Imported rice types usually are cheaper than domestic ones in Iran market and are consumed more widely.

### *Preparation of cooked rice grains*

In this research, two main cooking methods used for rice in Iran i.e. Kateh and Pilaw were applied.

10 grams of each sample were taken and washed in de-ionized water. Then in a beaker, 70 cc of de-ionized water was added to rice grains, brought to boiling and heated for 40mins. For Kateh method, after 40mins, heat was reduced until all the water evaporated, but for Pilaw method, after 40mins, heat was turned off and rice was separated from water by a drainer.

### *Sample analysis*

In this research, three treatments of rice were analyzed: raw rice grains and, cooked rice grains with the latter including Kateh and Pilaw types. 10 grams of each sample was dried at 110°C for 24 hrs. The samples were floured and 1 gram was taken and digested by a nitric-perchloric acid digestion method based on ASTM

standards in 2000 [18]. The samples were refluxed in a premixed solution of concentrated nitric acid and perchloric acid (70%) (3:1) at the rate of 20 ml per gram of sample, according to JahedKhaniki and Zazoli [17]. 2.5 ml sulfuric acid was added per gram of sample. After 30 mins, the mixture was put on the heater and the temperature gradually increased to boiling point. Heating was stopped when about less than 3 mL of a clear liquid was obtained. De-ionized water was added to bring the digest to 25 ml. The digested solution was analyzed for Pb, Cd, Cr, Ni and Co contents by atomic absorption spectrometer (ShimadzuAA-680 and Shimadzu MUV-IA). The samples were digested as triplicate and each sample was analyzed three times.

#### Quality control procedures

A rigorous quality control program was implemented, which included reagent blanks, replicated samples and a certified international reference material of SRM1568b (rice flour, National Institute of Standards and Technology, Gaithersburg, USA). The accuracy of the laboratory result was within the 87.5% confidential intervals of the stated reference values.

The mean blank value was deducted from the readings before the result was calculated. The batch blank was used to decide if the results of the batch were acceptable or not, i.e. if the blank was unacceptably high the batch was re-analyzed

### STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to evaluate statistical differences among the means of the heavy metal concentrations. One sample t-test was used to compare codex values with the obtained concentrations (the SPSS statistical package version 18.0).

### RESULTS AND DISCUSSION

The concentrations of the heavy metals in raw and cooked rice samples as means  $\pm$  standard deviation are

given in Table 1. Figure 1 compares the average contents of Cd, Pb, Cr, Ni and Co in three applied treatments (Raw, Kateh and Pilaw) on the three studied rice brands. One Way ANOVA (Duncan) analysis showed that the Cd, Ni and Co contents of the raw rice samples were significantly higher than the cooked samples in the three brands of rice. For Pb, a quite similar result was not observed; in Abdossaeid brand, the raw rice samples had significantly higher Pb content than the cooked samples did, whereas in the two other brands, there were no significant differences between raw and cooked samples. About Cr, the Pb results were reversed; there were no significant differences between raw and cooked rice samples in Abdossaeid brand whereas these differences in the two other brands were observed ( $p < 0.05$ ).

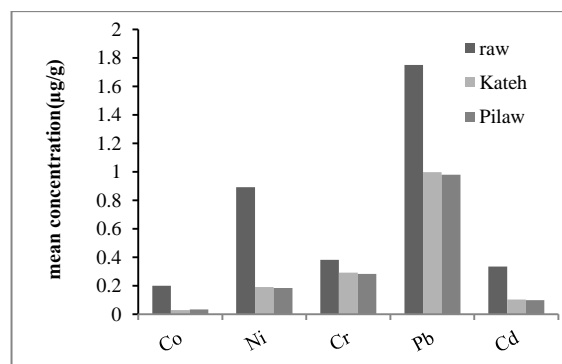


Figure 1. Mean concentrations of the detected metals in three treatments (raw, Kateh and Pilaw) for rice samples ( $\mu\text{g/g}$ )

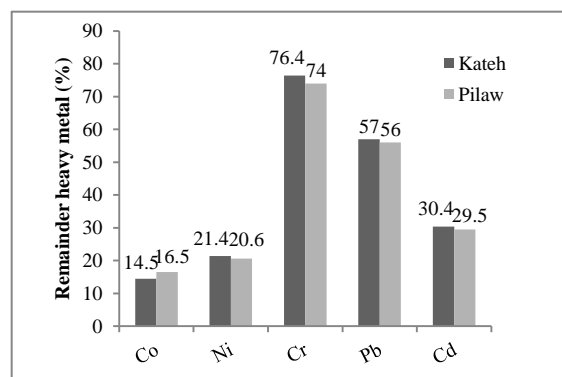


Figure 2. Remainder heavy metals after two methods of cooking (%)

There was no significant difference between the two ways of cooking rice in this study except for two cases

(in Abdossaeid brand for Co and Tajmahal for Cr) ( $p < 0.05$ ). Since in method pilaw, rice was pulled from the water, it seemed that rice samples Pilaw have less

metal contents than rice samples Kateh. This hypothesis was not observed in all samples (especially in Abdossaeid brand)

Table 1. The heavy metal contents (averages  $\pm$  Standard Deviation) in the three brands of rice (mg/kg, dry weight).

| brand |            | Treatment                      |                                |                                |
|-------|------------|--------------------------------|--------------------------------|--------------------------------|
|       |            | raw                            | kateh                          | Pilaw                          |
|       | Abdossaeid | 0.292 $\pm$ 0.018 <sup>a</sup> | 0.063 $\pm$ 0.017 <sup>b</sup> | 0.070 $\pm$ 0.039 <sup>b</sup> |
| Cd    | Maryam     | 0.346 $\pm$ 0.022 <sup>a</sup> | 0.096 $\pm$ 0.031 <sup>b</sup> | 0.088 $\pm$ 0.020 <sup>b</sup> |
|       | Tajmahal   | 0.365 $\pm$ 0.078 <sup>a</sup> | 0.146 $\pm$ 0.015 <sup>b</sup> | 0.140 $\pm$ 0.017 <sup>b</sup> |
|       | Abdossaeid | 2.008 $\pm$ 0.154 <sup>a</sup> | 0.522 $\pm$ 0.292 <sup>b</sup> | 0.696 $\pm$ 0.425 <sup>b</sup> |
| Pb    | Maryam     | 1.617 $\pm$ 0.371 <sup>a</sup> | 1.072 $\pm$ 0.321 <sup>a</sup> | 0.949 $\pm$ 0.331 <sup>a</sup> |
|       | Tajmahal   | 1.626 $\pm$ 0.180 <sup>a</sup> | 1.399 $\pm$ 0.267 <sup>a</sup> | 1.295 $\pm$ 0.418 <sup>a</sup> |
|       | Abdossaeid | 0.363 $\pm$ 0.161 <sup>a</sup> | 0.462 $\pm$ 0.050 <sup>a</sup> | 0.547 $\pm$ 0.371 <sup>a</sup> |
| Cr    | Maryam     | 0.395 $\pm$ 0.221 <sup>a</sup> | 0.215 $\pm$ 0.093 <sup>b</sup> | 0.217 $\pm$ 0.019 <sup>b</sup> |
|       | Tajmahal   | 0.384 $\pm$ 0.516 <sup>a</sup> | 0.195 $\pm$ 0.019 <sup>b</sup> | 0.083 $\pm$ 0.072 <sup>c</sup> |
|       | Abdossaeid | 0.906 $\pm$ 0.027 <sup>a</sup> | 0.145 $\pm$ 0.047 <sup>b</sup> | 0.158 $\pm$ 0.079 <sup>b</sup> |
| Ni    | Maryam     | 0.876 $\pm$ 0.087 <sup>a</sup> | 0.188 $\pm$ 0.054 <sup>b</sup> | 0.175 $\pm$ 0.065 <sup>b</sup> |
|       | Tajmahal   | 0.896 $\pm$ 0.035 <sup>a</sup> | 0.241 $\pm$ 0.025 <sup>b</sup> | 0.219 $\pm$ 0.058 <sup>b</sup> |
|       | Abdossaeid | 0.310 $\pm$ 0.014 <sup>a</sup> | 0.017 $\pm$ 0.027 <sup>b</sup> | 0.069 $\pm$ 0.027 <sup>c</sup> |
| Co    | Maryam     | 0.116 $\pm$ 0.057 <sup>a</sup> | 0.024 $\pm$ 0.042 <sup>b</sup> | 0.003 $\pm$ 0.006 <sup>b</sup> |
|       | Tajmahal   | 0.173 $\pm$ 0.059 <sup>a</sup> | 0.046 $\pm$ 0.043 <sup>b</sup> | 0.027 $\pm$ 0.034 <sup>b</sup> |

<sup>a, b, c</sup>Duncan comparison among concentrations in each row of the table. Different letters indicate significant differences.

Therefore we can say the type of rice cooking affects heavy metals reduction especially Cd, Ni and Co, but Pb and Cr should be tested with more samples, because in most samples of the cooked rice, the Pb and Cr contents decreased though not significantly. It cannot be stated which of the two cooking methods used in this study, resulted in a further reduction in the heavy metal contents, because in most cases, no significant differences were observed between them.

Zazouli and colleagues in 2010 [19] used the three treatments (raw, Kateh and Pilaw) for Iranian rice

cultivated in Babol region to detect Pb. They found that the average Pb concentration in samples of raw rice was higher than that in cooked rice (Kateh and Pilaw). Moreover, they detected lower Pb content in Pilaw than in Kateh in all samples. The first part of their results was similar to the results obtained in this research but some samples of the three studied rice brands did not confirm the second part of Zazouli and colleagues' results.

In 2012 Solidum and colleagues [20] measured Cr and Cd in different types of cooked rice grains available in the Philippine market; they prepared cooked rice samples near to Pilaw method of this research. They

detected the mean levels of Cr and Cd at 0.0006 and 0.0127 mg/kg respectively.

To clarify the status of the obtained concentration values better, the maximum allowable contents of metals in rice (published by Institute of Standards and Industrial Research of Iran (ISIRI) in 2010 [21]) were used in the current study. ISIRI has proposed 0.06 µg/g for Cd and 0.15 µg/g for Pb in rice. There were no permitted values available for the other heavy metals to be compared with those of the rice sample contents.

One sample t-test analysis showed that the Cd and Pb contents in the raw rice samples were significantly more than the values determined by ISIRI ( $p < 0.01$ ). This statistical analysis showed that the cooked rice samples had no significant differences with the permitted value of Cd in most samples but for Pb, almost 50% of cooked rice samples were significantly over the ISIRI codex. The results have been shown in table 3. These results declare that cooking of rice (regardless of cooking methods used in this study) can help us to achieve the permitted levels of Cd and Pb in rice grains.

Table 2. Comparisons of Cd and Pb contents in the different treatments of rice samples with ISIRI limit values for Cd and Pb in rice grains using one sample t-test

| treatment | Cd         |                           |                       |         | Pb         |                           |                       |         |
|-----------|------------|---------------------------|-----------------------|---------|------------|---------------------------|-----------------------|---------|
|           | Rice brand | Mean concentration (µg/g) | Standard value (µg/g) | P value | Rice brand | Mean concentration (µg/g) | Standard value (µg/g) | P value |
| raw       | Abdossaeid | 0.292                     | 0.1                   | <0.01   | Abdossaeid | 2.008                     | 0.2                   | <0.01   |
|           | Maryam     | 0.347                     | 0.1                   | <0.01   | Maryam     | 1.617                     | 0.2                   | <0.05   |
|           | Tajmahal   | 0.365                     | 0.1                   | <0.05   | Tajmahal   | 1.626                     | 0.2                   | <0.01   |
| Kateh     | Abdossaeid | 0.063                     | 0.1                   | >0.05   | Abdossaeid | 0.522                     | 0.2                   | >0.05   |
|           | Maryam     | 0.096                     | 0.1                   | >0.05   | Maryam     | 1.072                     | 0.2                   | <0.05   |
|           | Tajmahal   | 0.146                     | 0.1                   | =0.01   | Tajmahal   | 1.399                     | 0.2                   | <0.05   |
| Pilaw     | Abdossaeid | 0.070                     | 0.1                   | >0.05   | Abdossaeid | 0.696                     | 0.2                   | >0.05   |
|           | Maryam     | 0.088                     | 0.1                   | >0.05   | Maryam     | 0.949                     | 0.2                   | >0.05   |
|           | Tajmahal   | 0.140                     | 0.1                   | <0.05   | Tajmahal   | 1.295                     | 0.2                   | <0.05   |

Moreover, tolerable daily intake (TDI) amount is estimated for chemicals in food and drinking water based on body weight which can be daily received without any noticeable health hazards.

Toxic elements may accumulate in the body; therefore, the tolerable intakes have been represented on a weekly basis by Joint FAO/WHO Expert Committee on Food Additives (JECFA) under PTWI (Provisional Tolerable

Weekly Intake) standards to allow for daily variations in intake amounts of chemicals [22].

Estimated Daily Intake (EDI) through rice for heavy metals was calculated as follows:

$$EDI = \frac{C \times A}{Bw}$$

Where C is the metal concentration in rice sample, A is the amount of daily consumed rice in Iran and Bw is the average body weight (60kiloes).

The EDI values were compared with PTWI published by JECFA guidelines [23].For this comparison, EDI was multiplied by 7.

The obtained mean levels of Cd, Pb, Cr, Ni and Co for the raw, Kateh and pilaw rice samples have been shown in table 2. In this table, the respective EWI (Estimated Weekly Intake) values in three treatments of rice have been calculated (Iranian daily rice dietary is 110g per capita [21]).

Table 3.calculated values of EWI ( $\mu\text{g}/\text{kg}$  body weight) via eating rice and the published values of PTWI by FAO/WHO ( $\mu\text{g}/\text{kg}$  bw)

|        | Cd             |       |       | Pb              |        |        | Cr                |       |       | Ni              |       |       | Co    |       |       |
|--------|----------------|-------|-------|-----------------|--------|--------|-------------------|-------|-------|-----------------|-------|-------|-------|-------|-------|
|        | raw            | kateh | pilaw | raw             | kateh  | pilaw  | raw               | Kateh | pilaw | raw             | kateh | Pilaw | raw   | kateh | pilaw |
| EWI/60 | 4.286          | 3.914 | 1.270 | 22.458          | 12.808 | 12.577 | 4.889             | 3.734 | 3.619 | 11.460          | 2.451 | 2.361 | 2.567 | 0.372 | 0.423 |
| PTWI   | 7 <sup>a</sup> |       |       | 25 <sup>b</sup> |        |        | 23.3 <sup>c</sup> |       |       | 35 <sup>d</sup> |       |       | N*    |       |       |

\*: There is no PTWI set for cobalt but is used an MTDI (Maximum Tolerable Daily Intake) of 100  $\mu\text{g}/\text{kg}$  bw (= 700  $\mu\text{g}/\text{kg}$  bw in a week) for it [24].

<sup>a</sup>: [23, 25]

<sup>b</sup>: [25]

<sup>c</sup>: [16]

<sup>d</sup>: [26]

The limit values that have been determined in the PTWI for heavy metals, show the rate of health threat from any metal, i.e. the given smaller value indicates a higher risk of respective metal. These five heavy metals ranking as Cd> Cr >Pb > Ni > Co for the rate of health threat. According to figure 2, cooking process was less effective for reduction of the metals with higher hazard, while the process could be better for Co and Ni.

## CONCLUSION

The cooking process reduces the heavy metal contents in rice grains, while this process is not likely to be working similarly on other foods (According to the results of other studies). The present study showed that the rate of metal reduction after cooking was not equal for all of the metals and, the effect of cooking on the reduction of the studied metals was ranked from high to low: Co> Ni> Cd>Pb> Cr.

As shown in table 3 the weekly intake of Cd, Pb, Cr and Ni only through rice in the treatments of raw, Kateh and Pilaw were less than the PTWI values recommended by WHO/FAO, but intakes of these heavy metals will increase with consumption of other foods such as vegetables, meat, etc along a week. In this study cooking rice has reduced its rate of EWI. For example the weekly intake of Pb from the raw rice was 89.83% of PTWI, this percent decreased to 51.23 and 50.31% of PTWI with consumption of rice Kateh and Pilaw respectively.

The results showed that the cooking process was less effective for the more toxic metals. Therefore, the heavy metal health risks (especially the risk of Cd, Cr and Pb, with more toxicity) associate with the use of the studied imported rice brands.

The methods of rice cooking did not differ significantly in this study, in some cases, Kate process reduced more and sometimes Pilaw did.

The cooking process approaches the contents of heavy metals of rice grains to the permitted values of ISIRI significantly.

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