

Effect of precooking with sodium chloride and citric acid on residual amounts of lead and cadmium in rice

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Rice contamination with heavy metals has always been one of the main concerns of food health. The purpose of this research is to investigate the rice effect of the precooking process with sodium chloride and citric acid on the amounts of residual lead (Pb) and cadmium (Cd). In the current study, eight brands of high-consumption rice were used in the Arak-Iran market and their amounts of Pb and Cd were measured using graphite furnace atomic absorption spectrometry. Two rice brands, Pakistani Moeid and Indian 21, which contained the highest amounts of Pb and Cd, were selected and treated with soak (for 4h) and boiling (for 60 min) in 1% sodium chloride and 0.6% citric acid. The results showed that precooking Pakistani Moeid and Indian 21 rice samples with 1% NaCl reduced the amount of Pb by 15.7% and 16.2%, respectively. Furthermore, the amount of Cd was reduced by 10.5% and 10.8% for Pakistani Moeid and Indian 21, respectively. Furthermore, the results for precooking of Pakistani Moeid and Indian 21 rice samples with 0.6 % citric acid showed a reduction in the amount of Pb by 9.3% and the amount of 8.9% and Cd by 11% and 9.6%, respectively. These numbers were in comparison with precooking rice with pure water. According to the results of this research, precooking rice samples with NaCl reduces the amount of Pb more effectively than that of Cd. Furthermore, precooking rice with citric acid reduces the amount of Pb and Cd by almost the same amount. In general, precooking rice with NaCl is more effective in reducing heavy metal contamination compared to citric acid.

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Keywords

Rice, sodium chloride, citric acid, precooking, residual Pb and Cd

Introduction

The role of heavy metals in environmental pollution and their adverse effects on human health is clear to everyone (Nordberg et al. 2015). Heavy metal contamination of agricultural products is mainly due to anthropogenic activities, including agriculture mining, chemical fertilizers, irrigation by contaminated water, livestock manures, plant pesticides, and industrial processes (Yap et al. 2009). Among agricultural products, rice is a special product with a high accumulation of cadmium (Cd) (Meharg et al. 2013), and among various heavy metals, lead (Pb) is one of the most dangerous metals due to its potential toxicity to humans (Ashraf et al. 2015). Pb and Cd are heavy metals that do not perform an essential function in human physiology. It means that these elements are not necessary for body metabolism, and even small amounts of them are harmful to the body, and due to their long biological half-life, they can accumulate in body tissues (Morishita et al. 1987; Zarcinas et al. 2004). In fact, after entering the body, these elements are no longer excreted. They accumulate in some tissues such as muscles, bones, and joints and cause various diseases including neurological and respiratory disorders, abortion, liver, kidney, and brain damage (Bosque et al. 1990; Rezaian-Attar and Hesari 2014).

Rice is an important grain crop and a staple food for approximately 2.4 billion people around the world and is widely consumed in people's diets. According to the Food and Agriculture Organization (FAO), about 30% of the energy and 20% of the protein intake in the world are provided by rice (FAO 1993). Per capita, rice consumption in Iran is estimated to be 44.2 kg, which is the second most consumed product in the country, and today Iran is one of the largest importers of this product in the world (Noori 2007). Therefore, practical methods for reducing the amount of Pb and Cd in rice are of particular importance. One of the most convenient methods is to use various pre-cooking and cooking methods. So far, several studies have been conducted on the effect of washing, soaking, and cooking methods of rice in pure water on its reduction of Pb and Cd amounts (Adibi et al. 2014; Abbasi et al. 2021). However, there are some investigations available on the effect of precooking rice with sodium chloride and citric acid on the residual amount of Pb and Cd (Abu-Almaaly 2020; Pogson et al. 2020). Therefore, this study aimed to initially measure the concentrations of Pb and Cd in eight high-consumption rice brands distributed in Arak markets in 2020, and investigate the precooking effect of two rice brands, Pakistani Moeid and Indian 21, which contained the highest amount of Pb and Cd, with sodium chloride and citric acid on the residual amount of Pb and Cd.

Materials and Methods

Chemicals and Solutions

All chemicals, reagents, and standard solutions with maximum purity were purchased from Merck Company (Germany). Distilled water purified in a milli-q system (Milli-Q element, Millipore) was used for all solutions and dilutions.

Samples preparation

Eight brands of high-consumption rice grain named second crop Tarom, Amiri Tarom, black tail, Pakistani Moeid, Pakistani Shafa, Indian Mojdeh, Indian 21 and Uruguayan were collected in May 2020 from different market places in Arak, Iran. Based on the primary experiments, the Pakistani Moeid and Indian 21 brands contained the highest amount of Pb and Cd and were therefore chosen for further investigation. The raw rice samples (R) were simply washed three times with distilled deionized water (DDW) and allowed to dry initially at 110°C for 24 h and subsequently at 70°C to a constant weight, then milled to a fine powder and stored at 4° C before analysis. In cooking experiments 100 g of washed rice were soaked for 4 h in 700 ml; A) DDW (SW), B) 1% w/w sodium chloride solution (SS) and C) 0.6% w/w citric acid solution (SA) separately in a teflon pot. The

container was placed on a heater to achieve the boiling point and boiled for 15 min. The remaining water was discarded and the semi-cooked rice samples were washed three times with fresh DDW and heated 25 min in two stages. 5 min at high temperature to produce steam in the first stage and then 20 min at low temperature for the final cooking stage. The cooked rice samples were dried (similar to raw rice samples) and milled to a fine powder and stored at 4 °C prior to analysis.

Sample Analysis

We transferred 0.5 g of rice powder (raw or cooked) into Teflon reactors, then, 6 ml of concentrated nitric acid was added and digested in a microwave digester (Anton-Paar Multiwave 3000) in the 3 stages following (Table 1). The clear digested solution was transferred to polyethylene tubes and diluted to 100 ml with distilled deionized water and the Pb and Cd contents using a graphite furnace atomic absorption spectrometer (GTA 120 Varian).

No.	Power (w)	Time (min)	P	IP
1	450	15		
2	800	30	60 bar	240 °C
3	0	20		

Table 1. The conditions of microwave digester

All samples were digested 3 times, each sample was run 3 times, the coefficient of variation between three-time analyzes was less than 0.5 %. The contents of Pb and Cd were expressed as micrograms per kilogram of dry weight ($\mu\text{g}/\text{kg}$). The analytical procedure was checked using a spike recovery method (SRM), and the spike recovery for the metals was within 0.9 % of the actual values.

The calibration carved for Pb and Cd was linear in the range of 4-25 $\mu\text{g}/\text{kg}$ ($R^2=0.9991$) and 0.5-2 $\mu\text{g}/\text{kg}$ ($R^2=0.9986$), respectively. The detection limit (LOD) and the quantification limit (LOQ) for Pb were 1.2 $\mu\text{g}/\text{kg}$ and 4 $\mu\text{g}/\text{kg}$, respectively, and for Cd were 0.15 $\mu\text{g}/\text{kg}$ and 0.5 $\mu\text{g}/\text{kg}$, respectively.

Statistical Analysis

The mean and standard deviation of all quantitative data. Furthermore, we performed the ANOVA test to determine the significant variation in this difference of elements among the treated samples. We used a box-and-whisker plot to explore the variations of elements. All statistical analyzes were performed with SPSS ver. 16.

Result

The Indian 21 rice brand has the highest concentration of Pb and Cd among all rice samples; therefore, it was the first brand that was chosen for further research. Based on the results, the Cd concentration in the Pakistani Shafa rice brand is higher than that of the Pakistani Moeid rice brand, but the ANOVA test revealed that this difference is not significant (Table 2).

Brand	Pb	Cd
ANOVA	F=10.76, P= 0.000	F=6.890, P= 0.000
Correlations	p \leq 0.01, r=0.632	
Tarom (second crop)	100.1 \pm 18.6	10.4 \pm 3.5
Black tail	88.0 \pm 6.4	14.5 \pm 4.1
Tarom (Amiri)	97.0 \pm 13.8	10.1 \pm 4.2
Pakistani Moeid	185.3 \pm 13.4	39.2 \pm 4.6
Pakistani Shafa	94.6 \pm 19.1	42.7 \pm 6.8

Indian Mojdeh	122.1±12.9	24.1±4.2
Indian21	205.6 ±12.2	47.3 ±5.8
Uruguay	171.8±14.6	28.4±7.1

Table 2. Pb and Cd concentrations in eight brands of rice ($\mu\text{g}/\text{kg}$, dry weight)

Instead, the concentration of Pb in the Pakistani Moeid rice brand is much higher (about twice) than in the other; therefore, it was chosen as the second rice brand for further investigation (Fig. 1).

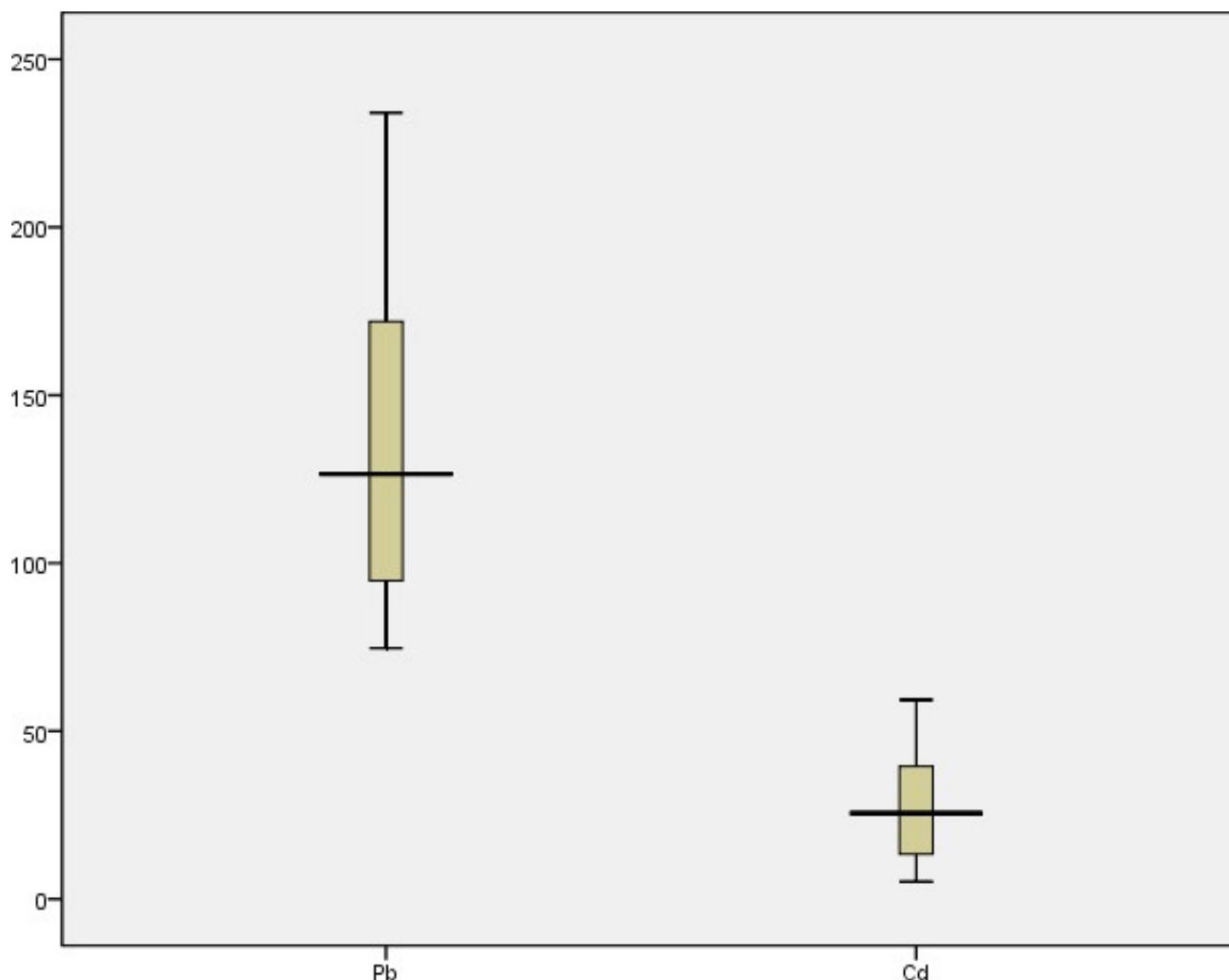


Figure 1. Box and whisker plot of Pb and Cd amounts in the evaluated brands.

The metal concentration in cooked rice samples decreased by 24.2% and 21.6% for Pb and 13.2% and 14.4% for Cd in the brands Pakistani Mood and Indian 21, respectively (Table 3).

	Pakistani Moeid		Indian 21	
	Pb	Cd	Pb	Cd
ANOVA test	$p \leq 0.01$	ns	$p \leq 0.01$	ns
Raw	185.3±13.4	39.2±4.6	205.6±12.2	47.3±5.8
Cooked (SW)	140.5±4.4	34.0±5.1	161.2±7.3	40.5±5.2
Reduction, %	24.2±2.4	13.2±12.8	21.6±3.6	14.4±7.5

Table 3. The concentrations ($\mu\text{g}/\text{kg}$, dry weight) of Pb and Cd in row and cooked rice samples in pure water (SW)

Naseri et al. (2018) measured the concentration of Pb and Cd in three imported rice available on the Shiraz-Iran market. They found that cooking Tajmahal rice grains reduces the content of Pb and Cd by 20.3% and 61.6%, respectively. Their results are consistent with those obtained in this study for Pb, but they are different for Cd. This discrepancy can be related to the difference in rice varieties (Morekian et al., 2014). Rezai-Malidareh et al. (2016) measured Pb and Cd in rice samples in west of Qhaem Shehr city paddies in northern Iran. They observed that the amounts of Pb and Cd in the cooked rice samples decreased by 16.9% and 14.7%, respectively, compared to the raw rice samples. These results are consistent with our finding. Liu et al. (2018) revealed that the amount of Pb in two Japanese and Indian rice samples decreased by 19.2% and 20% (after normal cooking), respectively.

Based on analyses performed with an X-ray fluorescence spectrometer, Mihucz et al. (2010) suggested that there is a layer of 80 μ thick on the surface of rice that depends on the type of the amount of metal, a fraction of metal in this layer is extracted in boiling water.

We also evaluated the effect of cooking rice samples in NaCl and citric acid solutions on the reduction of residual Pb and Cd. Therefore, after three times of washing with water, 100 g of raw rice samples were soaked in 700 ml of water, containing 8.1 g of of 1% NaCl (w / w) and 4.2 g 0.6 % citric acid (w/w) separately for 4 h. According to different experiments, we found that the optimal concentrations of NaCl and citric acid solutions to reduce the amounts of Pb and Cd in rice were 1% w/w and 0.6% w/w, respectively. The rice samples were cooked as previously described.

The concentrations (μ g/kg, dry weight) of Pb and Cd in raw rice samples, cooked rice samples in pure water (SW), in 1% NaCl (SS) and citric acid 0.6 (SA) are shown in Table 4.

Rice samples	Pakistani Moeid		Indian 21	
	Pb	Cd	Pb	Cd
ANOVA	$P \leq 0.000$	ns	$P \leq 0.000$	ns
Raw	185.3 \pm 13.4	39.2 \pm 4.6	205.6 \pm 12.2	47.3 \pm 5.8
Cooked in pure water (SW)	140.5 \pm 4.4	34.0 \pm 5.1	161.2 \pm 7.3	40.5 \pm 5.2
Cooked in NaCl solution (SS)	111.4 \pm 6.4	29.9 \pm 4.7	127.9 \pm 8.3	35.4 \pm 5.7
Cooked in citric acid solution (SA)	123.2 \pm 4.7	29.7 \pm 3.2	142.9 \pm 9.7	35.9 \pm 4.6

Table 4. *Pd and Cd concentrations (μ g/kg) in rice samples*

The content of reduction percentages of Pb and Cd in cooked rice samples, pure water (sw), 0.6% NaCl 1% (SS) and in citric acid solution (SA) compared to raw rice samples are presented in Table 5. The precooking of rice with NaCl reduces the amount of Pb and Cd in rice, but the reduction in the amount of Pb is greater than the amount of Cd. Abou-Almaaly (2020) investigated the effect of rice precooking with NaCl 2 % on residual lead and cadmium in different cooking methods. The method was not the same as that we utilized in the current study; meanwhile, the same results were obtained. Several studies have been conducted on the effect of treating fish with NaCl before cooking on reducing the concentration of mercury in it (Hajeb and Jinap 2009; Semenov et al. 2001). All studies indicate a decrease in mercury concentration in NaCl-treated fish.

Reduction percentages	Pakistani Moeid		Indian 21	
	Pb	Cd	Pb	Cd
Pure water (SW)	24.2 \pm 2.4	13.2 \pm 12.8	21.6 \pm 3.6	14.4 \pm 7.5
NaCl solution (SS)	39.9 \pm 3.5	23.7 \pm 11.9	37.8 \pm 4.0	25.2 \pm 11.9
Citric acid solution (SA)	33.5 \pm 2.5	24.2 \pm 8.2	30.5 \pm 4.8	24.0 \pm 9.7
Net NaCl	15.7	10.5	16.2	10.8
Net citric acid	9.3	11.0	8.9	9.6

Table 5. Reduction percentages of Pb and Cd contents of cooked rice samples compared with row rice samples

Amiard et al. (2008) showed that boiling soft tissues in NaCl reduces their amounts of Pb and Cd. They believed that it was attributed to the outflow of water from the tissue while boiling, and along with the water removed from the tissue, Pb and Cd also come out.

We found that the reduction of the amounts of Pb and Cd in rice while precooking with NaCl is due to two reasons; first, the ion exchange of high-concentration sodium ions (about 0.2 m) in solution with low concentrations of Pb and Cd ions in rice at 100 °C. The second reason is the electrostatic attraction of chloride anions in solution with Pb and Cd cations in rice, and this attraction for Pb ions is higher than that of Cd ions.

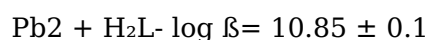
The solubility of leaf chloride in 100 °C water is approximately 0.12 M, while for cadmium chloride it is approximately 0.8 M, which means the attraction of chloride ions with lead ions is approximately 6.5 times higher than that of cadmium ions.

This could be the reason why there is a greater reduction in the amount of Pb in rice samples than that of Cd due to cooking in NaCl solution. According to Table 5, rice precooking with citric acid also reduces the amount of Pb and Cd in it, but there is no significant difference between the reduction of Pb and the reduction of Cd.

Many studies have been conducted in the reduction of heavy metals from food staff by treating them with chelating against (Abu-Almaaly 2020; Hajeb and Jinap 2012; Aizpura et al. 2003).

Citric acid is one of these substances that can form a stable complex with heavy metals. Semenov et al. (2001) used 0.1 % citric acid to remove heavy metals from the fish. Hajeb and Jenap (2012) used a solution of cysteine, EDTA, and NaCl to reduce the amount of mercury in fish tissue by up to 91%. Behrouzi et al. (2020) were able to reduce the amount of arsenic in rice by up to 80% using a solution of 1% citric acid. Abou-Almaaly (2020) used 2% citric acid to reduce the amount of Pb, Cd, and As in rice. Pogoson et al. (2020) used 1M citric acid with 1m calcium carbonate to reduce the amount of cadmium in rice by up to 79%.

The functional hydroxyl and carboxyl groups in the citric acid molecule can form a chelating complex with Pb and Cd ions in rice and extract them to solution and reduce their amounts in rice (Hajeb et al. 2014). Citric acid (H₃L) is a tricarboxylic acid with three dissociation constants such as PK: 3.13, 4.76 and 6.4. Therefore, it is mainly found in water such as H₃L and H₂L⁻. According to the following data, citric acid can form a stable complex with Pb and Cd:



Also, as mentioned above, the stability constant of the Pb ion complex is almost the same as that of the Cd ions. Therefore, the lack of a significant difference between the reduction amounts of Pb with the reduction amount of Cd in rice is justified, because of the precooking of it with citric acid.

Conclusion

The results reveal that precooking with 1% NaCl reduces the amounts of Pb by 39.9% and the amounts of 37.8% and Cd by 23.7% and 25.2% for the Pakistani Moeid and Indian 21 rice samples, respectively. Furthermore, precooking rice with 0.6% citric acid reduces Pb to 33.5% and 30.5%, and Cd amounts to 24.2% and 24.1% for Pakistani Moeid and Indian rice samples, respectively. Therefore, in cases where rice is contaminated with Pb, it is recommended to wash row rice three

times with water and soak it in 1% NaCl for four hours, then semi-cook it with the same NaCl solution, rinse it again with water, and finally cook it. The precooking rich with NaCl has a greater effect on reducing the amount of Pb than the precooking rich with citric acid. Rice precooking with NaCl or citric acid has a similar effect on the amount of Cd residue. Obviously, the water, NaCl, and citric acid used should not be contaminated with Pb or Cd.

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