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THE EFFECT OF DIFFERENT LEVELS OF SODIUM CHLORIDE ON METAL RESIDUES (Cd, Zn, As and Cr) DURING COOKING OF IRANIAN AND INDIAN RICE CULTIVARS

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ABSTRACT

Rice is a cereal grain consumed as the staple food in a large part of the world. Its plant requires plenty of water and sunshine. Rice cultivation dates back to several centuries ago, as the agricultural history shows that its cultivation was practiced during the Achaemenid Empire. The main objective of this study was to examine the effect of sodium chloride on reducing heavy metals (As, Cd, Zn and Cr) in Tarom Hashemi and Indian rice cultivars. Accordingly, heavy metals were first quantified in raw Tarom and Indian samples. Different levels of sodium chloride (1, 1.5, 2, 2.5 and 3 g) were then added to boiling rice samples, which were then drained, milled and dissolved to obtain a transparent solution. Finally, the level of different heavy metals was determined by Atomic Absorption Spectroscopy (AAS). Results showed that added salt reduced heavy metal levels in both cultivars, especially in H3 and T3 treatments, where the lowest heavy metal levels were observed. Moreover, in all treatments, their levels were below the recommended standard. In other words, NaCl was effective in reducing heavy metal levels.

Keywords: Boiling, Rice, Arsenic, Cadmium, Zinc, Chromium

INTRODUCTION

Rice is one of the most consumed grains in the world and is extensively included in food diets. FAO statistics for 2004 show that rice provides for 30% of the potential energy and 20% of the protein diet requirements of people worldwide. The pollution of soil and aquatic environments with heavy metals is a serious growing problem. Human-released toxic metals have polluted most pieces of land as, in some cases, the pollutions are above normal limits. Almost all metals leave harmful effects on human body, however the effect of Cd, Zn, As and Cr is more significant as they cause numerous health-related problems including renal failure, pulmonary problems and cancer[1]. Recent investigations show that cereals, rice products, tea, root vegetables and seafood harbor high levels of arsenic[2]. Regarding Cd, it can be said that once entering the human body, it starts to accumulate in organs, particularly in liver and kidneys, and when it exceeds specific thresholds, emerges in form of various diseases due to its long-lasting adverse effects. These complications are more intense in children. Rice growers use chemical fertilizers to boost their yields. This can contribute to the accumulation of these metals in rice. Agricultural products (*e.g.* rice and wheat) and seafood are prone to cadmium pollution due to soil and water pollutions[3]. Although in small quantities, table salt has always been present in food diets of all classes of society. Therefore, any pollution of salt, even in trace amounts, can pose threats to consumer health. Traditionally, salt has been a flavoring, preservative and taste booster agent. Today, salt is being used in the food industry cost-effectively and with high availability. The Food Safety Authority Of Ireland (FSAI)[1] and Food Standard Agency (FSA)[2] recently reported that, in the UK, the daily intake of sodium through table salt is 3.3-3.9. The large quantities of salt used in processed foods have recently raised concerns for the health of their consumers. The high consumption of salt in Iran can be due to the fact that the use of refined salt has not been publicly promoted yet, and most marketed salts are unrefined salts with large amounts of impurities that cover the salty taste[4]. Cooking is a major stage of food preparation comprised of mass transfer and heat transfer. About 90% of the dry weight of milled rice is starch[5]. Rice grains absorb moisture during cooking and thus expand as a result of gelatinization. The texture of cooked rice

[1].Food Standard Agency
[2].Food Safety Authority Of Ireland

grains is not a simple property, rather it is a combination of multiple properties including hardness, adhesiveness and chewiness. Cooking methods and tastes about cooked rice texture vary by place. Western consumers of rice prefer long-grained, puffy and cooked-flavor rice[6].Indians like medium-grained, puffed, cooked-flavor rice with a soft kernel, whereas Japanese prefer their cooked rice being soft and sticky[7]. In Iran, long-grained, well-whitened rice with good fragrance and longer length when cooked are preferred. Like the Sadri cultivar, different Tarom rice cultivars are among the best rice cultivars and their origin is in Mazandaran, Iran. The latter is slightly shorter and fatter than Sadri, and keeps its good taste and fragrance for hours after being cooked. Moreover, similar to Sadri grains, when rubbing Tarom grains between both palms, their fat can be felt. Tarom Hashemi rice accounts for the largest cultivation area, thanks to its desirable appearance, high marketability, easy cooking and good. Therefore, the state of the problem includes the effect of different levels of sodium chloride on metal residues (Cd, Zn, As and Cr) during boiling of Iranian and Indian rice cultivars. The existence of heavy metals in modern industries has turned into a serious concern as these elements can be introduced into the human food chain by various means, thus affecting human development and health[8]. Usually, plants intake soil salts that contain metals. Rice is no exception to this reality and it can be polluted by heavy metals such as zinc, cadmium, arsenic and chromium during its growth stages. These metals can enter human body by consuming polluted rice. Heavy metals do not become metabolized in the human body. In fact, they would never leave once they enter the body and accumulate in organs. This causes numerous diseases and complications including spread of viral, bacterial and fungal infections. Moreover, these elements can replace other substances and salts needed by the body. These elements precipitates in vessel, muscular, bone and joint tissues. Poisoning with heavy metals can cause neurological disorders, cancers, malnutrition, hormonal imbalance, abortion, respiratory and cardiovascular disorders, damages to liver, brain and kidneys, allergies, anorexia, progeria, memory loss, hair loss, osteoporosis, insomnia, weakened immune system, anemia, gene damage and even death[9]. Over-intake of these metallic elements can lead to poisoning along with other disorders like renal failure, cirrhosis of the liver and disorders in RBC production. Heavy metals like Cd and Zn are environmental pollutants that can enter human body through various vehicles. Accordingly, the International Agency for Research on Cancer[3] (IARC) introduced cadmium as a carcinogenic agent and a cause of renal failure. It is also effective in poisoning and damages to the texture of liver, heart, kidney and sexual glands. Zinc poisoning is a prevalent environmental pollution in the US and a health threat in Iran. Zinc interferes with the function of body organs and leaves adverse effects on nervous system, and hepatic and renal texture. Additionally, it contributes to hearing impairment, weakened immune system, learning disability, low birth weight and preterm birth[10]. Arsenic is an unnecessary mineral for plants and are classified as toxic for them[11]. Release and absorption of As vary by the type of poisoning, as acute poisoning occurs in liver and kidneys, and chronic poisoning is focused on hairs and nails. Arsenic interferes with phosphate metabolism in plants and disturbs cellular functions by reacting with sulfhydryl groups of enzymes and textural proteins, leading to cell death. Six-valent chromium (Cr^{6+}) is used in producing lung cancer drugs and treating skin conditions. Recent research shows that a balanced amount of Cr in animal diets enhances their life. Chromium is available in 3-valent chromites. This type of Cr can be solved in and transported by water. Therefore, the adverse effects of chromium-polluted water are not detectable around regions with high Cr reserves. Skin burns, skin irritations, respiratory conditions, gradual failure of kidneys, liver, stomach and intestine (digestive system) and different types of lung cancer are among the prevalent signs of human body intoxication with chromium [12]. The most important effective factor in cooked rice quality depends on properties of starch that constitutes 90 of white dry rice. Therefore, an evaluation of starch properties would be important for predicting the quality of cooked rice. Different consumers hold different opinions about the important of grain size and shape. The research rationale focuses on the effect of NaCl salt on residual heavy metals (Cd, Zn, As and Cr) of Iranian and Indian rice cultivars. Hedayaty *et al.* (2010) studied the popular rice cultivars of Lorestan, Iran, and showed that the zinc and cadmium levels were 77 and 37 ppb, respectively[13]. Given the high consumption of Indian rice cultivars in Iran and also the health significance of determining Zn and Cd levels of rice, Malakootian *et al.* (2011) carried out a study on this rice. First, twenty different varieties

[3].International agency research cancer

of popular Indian rice were purchased from the Iranian market, taking three samples from each brand. The samples were acid-digested, and their Cd and Zn levels were measured by atomic absorption spectroscopy. Their results showed no Cd in the samples as its level was lower than the detection threshold of the devic (0.015 mg/kg). The zinc level was however slightly higher than the FAO/WHO recommendations[1]. In a study on rice cultivated in Shahrekurd, Iran, Shakerian *et al.* (2012) reported that Zn and Cd level were lower than the allowable standard . Abdulrahman *et al.* (2008) investigated various fish species of the Persian Gulf. They reported that zinc levels in most fish species were lower than <0.02 µg/g. However, different cooking methods left small effects on zinc content, whereas cadmium levels remained the same through all cooking methods [14]. Domingo *et al.* (2008) randomly collected different foodstuffs from local groceries and markets. The purchased foods included fish, meat, legume, potato, rice and olive oil. Heavy metal levels were first measured in raw foods. Samples were then cooked using multiple methods (including boiling, frying, grilling and roasting), whereas the rice samples were cooked only by boiling for 29 minutes at 68°C. Cd and Zn levels in raw rice samples were very low. Cooking had no considerable effect on these metals in rice. However, boiling other foods slightly reduced their levels.

MATERIALS AND METHODS

The studied population included treatments with 1, 1.5, 2, 2.5 and 3% (w/w_{rice}) NaCl salt. No salt was added to the control treatment. Table (1) presents the study treatments.

Table 1 - Study treatments

Treatment	Code
Boiling Tarom Hashemi rice, with 1% NaCl	H_1
Boiling Tarom Hashemi rice, with 1.5% NaCl	$H_{1.5}$
Boiling Tarom Hashemi rice, with 2% NaCl	H_2
Boiling Tarom Hashemi rice, with 2.5% NaCl	$H_{2.5}$
Boiling Tarom Hashemi rice, with 3% NaCl	H_3
Boiling Tarom Hashemi rice, without NaCl (control)	H
Raw Tarom Hashemi rice (control)	H_0
Boiling Indian rice, with 1% NaCl	T_1
Boiling Indian rice, with 1.5% NaCl	$T_{1.5}$
Boiling Indian rice, with 2% NaCl	T_2
Boiling Indian rice, with 2.5% NaCl	$T_{2.5}$
Boiling Indian rice, with 3% NaCl	T_3
Boiling Indian rice, with without NaCl (control)	T
Raw Indian rice (control)	T_0

Based on the experiment method, since there were 15 treatments and 3 replications, a total of 45 experiments were conducted on Iranian and Indian rice cultivars. Experiments included measurements of As, Zn, Cd and Cr heavy metals using an AAS instrument and the hydride generation (HG) technique. Given the number of treatments, about 2 kg Tarom Hashemi and 2 kg Indian rice and 100 g refined salt were required. Samples were then prepared for measuring the heavy metal content. To avoid any unwanted contamination, all containers were acid-washed, were rinsed with deionized-distilled water, and were dried in an oven. From each rice sample, 2g samples were exposed to 105°C for 48h. In the next step, 10ml of perchloric acid (70%), 5ml of sulfuric acid and 30ml of nitric acid (70%) were added to the samples, which were then kept at room temperature for half an hour. They were then slowly heated to come to a boil, giving 3ml of a transparent stable solution, which was then brought to 25ml using deionized-distilled water. In the said study, first two measures of water were added to a 600cc beaker, to which 100g rice (weighed by a precise balance with ±0.0001g repeatability) was added. The mixture was first heated on a heater at 120°C for 15 minutes, to let the water come to a boil. Different salt levels (1, 1.5, 2, 2.5 and 3 g, already weighed) were added to the water-rice mixture, letting the mixtures boil for another 5-6 minutes. The mixtures were then drained by a plastic colander, and left for 5 minutes to let the solution fully be drained. Rice samples were then placed in watch glass oven-dried for 6 hours at 105°C until reaching a constant weight. At this point, 2g rice was completely milled in a Moulinex food mill, which were then kept in plastic bags and were left at room temperature until performing experiments at 4°C. The 2g powdered rice was added into a 100cc beaker and was heated at 105°C. Beneath a fume hood, 10ml of perchloric acid (70%) was added to each sample, and the mixture was heated slowly beneath the fume hood for 15 minutes to let it slowly boil. This was continued until 3-6 ml of the mixture was left, to which 5ml sulfuric acid was added. The result was slowly reheated under the fume hood for another 15 minutes and were left to boil smoothly until 3-6 ml was left. At this point 30 ml of nitric acid (70%) was also added, and a watch glass was placed upon the beaker (due to the added nitric acid, the solution becomes yellow, foams and thus release N_2 during boiling). The heater was set at 105°C for 3-4 hours until the solution became completely transparent. Once the solution reached down to 10-15 ml, the solution was poured into a 100 ml Erlenmeyer flask and was reached to 25 ml using deionized-distilled water. The cool solution was then poured into conical tubes (Falcon), 3cc of each sample was taken to be tested by contrAA 700 AAS instrument and the HG technique (made in Germany) in order to determine heavy metal contents of In order to determine Cd, Zn, Cr and As levels using the AAS instrument, the following wavelengths were selected: 228.8 nm (Cd), 283.3 nm (Zn), 357.9 nm (Cr) and 193.7 nm (As).After adding the standards and samples to the graphite furnace AAS instrument, their absorption levels were measured. These absorption values were then converted into concentration levels using the calibration curve. After applying the data factors, the final concentrations of these metals in samples were determined by ppb.

rice. Figure 3.1 and 3.2 present the powdered Tarom Hashemi and Indian rice samples and also their prepared solutions for AAS-analysis of heavy metals. The procedure for determining heavy metal contents using the AAS and graphite furnace techniques is discussed. Analysis of heavy metal contents by graphite furnace AAS

Statistical Analysis

Given the experimental treatments, data were analyzed through a completely randomized design with three replications. SAS 19 and Excel 2013 were used for this purpose. The comparison of means and significance tests were performed using one-way ANOVA and Duncan's multiple range test.

RESULTS & DISCUSION

Results of mean comparisons for heavy metals of Tarom and Indian rice cultivars were showed in table 2.

Table2-Results of mean comparisons for heavy metals of Tarom and Indian rice cultivars (ppm)

Treatment	Mean As (ppm)	Mean Cd (ppm)	Mean Zn (ppm)	Mean Cr (ppm)
H_0	0.23 ± 0.001^{a}	0.092 ± 0.001^{a}	0.237 ± 0.002^{a}	427.8 ± 30^{b}
H	0.223 ± 0.002^{ab}	0.09 ± 0.002^{b}	0.236 ± 0.001^{a}	420 ± 22^{c}
H_1	0.2 ± 0.002^{cd}	0.08 ± 0.002^{d}	0.205 ± 0.002^{d}	285.65 ± 24^{f}
$H_{1.5}$	0.18 ± 0.001^{e}	0.075 ± 0.002^{ef}	0.189 ± 0.002^{e}	28 ± 4.245^{h}
H_2	0.16 ± 0.002^{f}	0.07 ± 0.001^{fg}	0.175 ± 0.003^{f}	240.2 ± 26^{hi}
$H_{2.5}$	0.15 ± 0.002^{gh}	0.065 ± 0.002^{gh}	0.161 ± 0.002^{g}	27 ± 8.226^{j}
H_3	0.145 ± 0.002^{hi}	0.06 ± 0.002^{hi}	0.146 ± 0.001^{ij}	198.6 ± 26^{i}
T_0	0.225 ± 0.002^{ab}	0.091 ± 0.003^{b}	0.22 ± 0.002^{b}	410 ± 24^{d}
T	0.22 ± 0.001^{b}	0.085 ± 0.001^{c}	0.21 ± 0.001^{bc}	400 ± 28^{e}
T_1	0.195 ± 0.002^{d}	0.078 ± 0.001^{de}	0.20 ± 0.002^{d}	256.2 ± 24^{g}
$T_{1.5}$	0.16 ± 0.001^{f}	0.071 ± 0.001^{f}	0.185 ± 0.001^{e}	24 ± 5.238^{i}
T_2	0.15 ± 0.003^{gh}	0.063 ± 0.001^{h}	0.170 ± 0.003^{f}	25 ± 5.236^{i}
$T_{2.5}$	0.14 ± 0.003^{i}	0.06 ± 0.003^{hi}	0.155 ± 0.001^{h}	51.211 ± 21^{k}
T_3	0.13 ± 0.002^{j}	0.058 ± 0.001^{i}	0.14 ± 0.003^{j}	89.175 ± 21^{m}

According to Duncan's test, means with at least one similar letter have no significant difference at 0.01%

Evaluation of NaCl effect on heavy metal levels of Tarom and Indian rice cultivars

Results in Table (2) show that boiling rice samples without salt and raw rice samples from both cultivars had significant differences with other treatments (T_1,$T_{1.5}$,T_2,$T_{2.5}$,T_3, H_1,$H_{1.5}$,H_2,$H_{2.5}$, and H_3). However, there was no difference between H_0 and H as well as T_0 and T. A closer look at the table reveals that adding the NaCl salt reduces arsenic concentration, as higher levels of NaCl caused higher reduction of As in rice. Additionally, simultaneous cooking of rice with salt further reduced the levels of this metal in rice samples. This can be due to the solubility of arsenic in brine, and depends on the water temperature, salt concentration and salt type. In this study, the cooking conditions and NaCl salt contributed to the reduction of As levels in rice samples. These results were in agreement with those reported by Bea *et al.* (2002) who suggested that As contents are different in raw and cooked rice samples. They used an atomic absorption spectroscopy with hydride generation (AAS-HG) to measure metal levels, and reported that the cooking process is effective in reducing as content of raw rice[15]. Islam *et al.* (2004) also suggested that the cultivated tice were contaminated with As, considering its high 2 μg/g level[16]. They also reported that As levels were different in parboiled and raw rice samples. Accordingly, its level was 6.59 lower in cooked rice than raw rice samples. They explained this can be due to the mechanisms occurring during cooking. suggested that As reduction during cooking can be due to increased solubility of metals at higher water temperatures. Devesa *et al.* (2001) claimed that temperatures higher than 100°C cause reduction in the levels of metallic elements in food samples due to their decomposition at these temperatures[17].

Evaluation of NaCl effect on Cd levels of Tarom and Indian rice cultivars

Table (2) shows that boiling rice samples without salt and raw rice samples from both cultivars had significant differences with other treatments (T_1,$T_{1.5}$,T_2,$T_{2.5}$,T_3, H_1,$H_{1.5}$,H_2,$H_{2.5}$, and H_3). Moreover, there were significant differences between H_0 and H as well as between T_0 and T. A closer look at the table reveals that adding the NaCl salt reduces the cadmium level, as higher levels of NaCl caused higher Cd reduction in rice samples. Additionally, simultaneous cooking of rice with salt further reduced the levels of this metal in rice samples. This can be due to the solubility of cadmium in brine, and depends on the water temperature, salt concentration and salt type. In this study, the cooking conditions and NaCl salt contributed to the reduction of Cd levels in rice samples. Authors have analyzed the effect of the cooking process on the amount of heavy metals and found that, firstly, increasing the cooking temperature improves the reduction of heavy metal levels, and it was especially effective in reducing Cd, Zn and Pb levels. Secondly, adding NaCl salt had a 10-15% contribution in reducing the levels of heavy metals. Thirdly, the use of other salts like sodium bicarbonate (also known as baking soda) as a neutralizer is also effective in reducing heavy metal concentrations [18].

Evaluation of NaCl effect on Zn levels of Tarom and Indian rice cultivars

Results in Table (2) show that boiling rice samples without salt and raw rice samples from both cultivars had significant differences with other treatments (T_1,$T_{1.5}$,T_2,$T_{2.5}$,T_3, H_1,$H_{1.5}$,H_2,$H_{2.5}$, and H_3). Moreover, there was no significant difference between H_0 and H as well as between T_0 and T. Additionally, simultaneous cooking of rice with salt further reduced the zinc level in rice samples. This can be due to the solubility of zinc in brine, and depends on the water temperature, salt concentration and salt type. In this study, the cooking conditions and NaCl salt contributed to the reduction of Zn levels in rice samples. Studies by other researchers also showed that rice washing and cooking are highly effective in eliminating its heavy metals. That is, washing and cooking are highly effective in reducing heavy metals of rice samples.

Evaluation of NaCl effect on Cr levels of Tarom and Indian rice cultivars

Results in Table (2) show that boiling rice samples without salt and raw rice samples from both cultivars had significant differences with other treatments (T_1,$T_{1.5}$,T_2,$T_{2.5}$,T_3, H_1,$H_{1.5}$,H_2,$H_{2.5}$, and H_3). Moreover, there were significant differences between H_0 and H as well as between T_0 and T. These treatments had the highest chromium content compared to other treatments. Chromium contents of all treatments, however, were lower than the standard level (S treatment) (< 1 ppm). In this study, the combination of NaCl salt and cooking managed to reduce chromium content in rice samples. This can be as a result of Cr solubility in water, whereas high cooking temperatures were also effective in increasing its solubility and elimination from rice samples. Results were consistent with those reported by Lin *et al.* (2004) who suggested that Thai rice samples had Cr contamination equivalent to 0.01 mg/kg within the standard range [19].

CONCLUSION

Study findings suggest that adding NaCl contributes to reducing As, Cd, Zn and Pb in Tarom and Indian rice cultivars during cooking. According to the results, the levels of As, Cd, Zn and Cr in Tarom samples with 3% salt were reduced by 0.13, 0.058, 0.14 and 175.89 ppm, respectively. For Indian samples with 3% salt, these reductions for As, Cd, Zn and Cr were 0.145, 0.06, 0.146 and 198.6, respectively. The levels of these metals in all the above-mentioned treatments (boiling with 3% salt) were lower than the recommended standard and other treatments and samples. In other words, it can be concluded that application of higher levels of NaCl, along with cooking, can further reduce heavy metal concentrations in rice samples.

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