

Analysis of arsenic in raw and cooked rice by atomic absorption spectrophotometer

Jansen Silalahi¹, Sanggam Dera Rosa Tampubolon^{2,*}, Margareth Ruth Mayria Sagala³, Icha Nanda Saraswati¹ and Yosy C E Silalahi⁴

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara Medan Indonesia

²Faculty of Agriculture, Universitas Katolik Santo Thomas Indonesia

³Department of Environment of Health, Faculty of Public Health, Universitas Sumatera Utara Medan Indonesia

⁴Departement of Pharmacy, Faculty of Health Sciences, Universitas Sari Mutiara, Medan Indonesia

* E-mail: rosatampubolon031969@gmail.com

Abstract. Arsenic is a highly toxic and carcinogenic metalloid. Rice is a staple food that is widely used in the world, especially in Indonesia. Several studies have shown that arsenic level in rice is influenced by the environment and genetic differences of rice. The process of cooking in raw rice may reduce arsenic level in rice. The purpose of this research was to study the effect of cooking process using demineralized water and tap water on arsenic contents in rice. The samples used were white rice (*Oryza sativa* L), brown rice (*Oryza rufipogon*), “brown” rice (*Oryza punctata*) and black sticky rice (*Oryza sativa* var *glutinosa*) obtained from several traditional markets in Medan. The process of cooking was done using two types of water, namely demineralized (reverse osmosis; RO) water and tap water. The determination of arsenic level was carried out using atomic absorption spectrophotometer with argon-air flame at wavelength of 193.7 nm. The results showed that the highest arsenic content of 0.3061 mg/kg was found in red rice and the lowest arsenic level of 0.0693 mg/kg was found in white rice. Process of cooking with RO water and tap water reduced arsenic level in cooked rice. The highest reduction in arsenic content (21.93%) caused by cooking process using RO water and 11.40% caused by cooking with tap water. The result showed that the arsenic safety index in raw rice and cooked rice, based on the daily intake according to the excess cancer risk, exceeded the WHO safety standard.

Keywords: arsenic, cooked rice, reverse osmosis, tapwater, atomic absorption spectrophotometer

1. Introduction

Arsenic (As) is a highly toxic metalloid. In nature, arsenic is commonly found as organic and inorganic compounds. The international agency of research on cancer (IARC) states that arsenic is the first group of carcinogens [1,2]. Rice is a staple food commonly consumed by people all over the world, especially in Asia. Indonesia is a country with the second largest rice consumption in the world after Bangladesh. Rice



is also a source of protein in rural communities [3]. Generally, the white rice is more frequently consumed than the other rice varieties such as brown rice and black rice [4].

Research conducted by WHO reported that rice was the main source of arsenic exposure for humans [5]. Rice is an anaerobic plant. In anaerobic conditions, arsenic in the soil was absorbed causing arsenic level in rice to be about 10 times higher than in other plants. Arsenic levels in rice grains increased higher when planted in soil contaminated with arsenic, such as when the farmers use of pesticides, and irrigation water contains high arsenic level. FSA and Duxbury et al. showed revealed that arsenic levels in rice ranged from 0.001 mg/kg to 0.42 mg/kg [6,7]. The arsenic level found in rice in the average of 0.14 µg/kg was reported by [8]. In research conducted by the *Food Drug Administration* in 2012 to 2013 on 200 samples of rice and rice products, it was found that the arsenic content was higher in brown rice than in white rice. In a study investigating the arsenic level in all rice circulating in America found that all rice contained arsenic at various levels ranging from 0.0171 µg/kg in white rice to 0.963 µg/kg in red rice [9]. Research with the determination of arsenic levels in rice and food had been widely conducted in many countries; the results obtained showed that the arsenic level was worrying to the public health because it exceeded the safe level (0.3 mg/kg) for rice standardized by the [10].

Pal et al. showed that the cooking process was able to reduce arsenic level as much as 8% -58% [11]. Sengupta et al. also reported that the cooking process using water with low arsenic levels was able to reduce arsenic levels up to 28% when using tap water, well water, and rainwater [12]. Similarly Halder et al. showed that cooking process with tap water was able to reduce arsenic level as much as 7.5% to 66.3%. However, the process using well water was able to increase arsenic level (4.1%) due to the high arsenic content in the water used for cooking [13]. Determination of arsenic content in rice in Indonesia had not been done yet. The aim of this study was to study the effect of cooking process using RO water and tap water on the arsenic level in different type of rice. Arsenic determination in this study used atomic absorption spectrophotometer [14].

2. Materials and methods

The study was conducted at the Laboratory of the Central Agro Industry in Bogor, West Java in 2017. The tools used were glass wares, blenders, rice cookers, Whatmann No.41 papers, analytical balance (Mettler Toledo), microwaves (SCP Science), atomic absorption spectrophotometer (GBC 906AA) with air-argon with arsenic lamp cathode. The materials used in this study were tap water, RO water and E.Merck pro analysis grade materials, namely 65% nitric acid, arsenic standard 1000 µg/mL, and 37 % hydrochloride acid.

The samples used in this study were rices obtained from several traditional markets in Medan, Indonesia. The types of the tested rice were white rice (*Oryza sativa* L), red rice (*Oryza rufipogon*), brown rice (*Oryza punctata*) and black rice (*Oryza sativa* var *glutinosa*). As much as 100 g of raw rice was washed with flowing water and drained to dry. Then this washed rice was analyzed for arsenic content. One hundred and fifty (150) g of rice were washed 3 times with tap water and RO water. Then, the rice was put into the rice cooker and cooked using tap water and RO water, where the ratio of rice to tap water or RO water was 1:2. After cooking, it was cooled and each 100 g of them was weighed and blended.

A 10 mL of arsenic standard solution (1000 µg/mL) was pipetted and then transferred into a 100 mL volumetric flask and filled to the mark line with distilled water (concentration of 100µg/mL). From this solution a 5 mL was pipetted then transferred into a 500 mL volumetric flask and filled it up to the mark line with distilled water (1 µg/mL). From this solution, (0, 0.5, 1, 1.5, 2, 2.5) mL were pipetted and then transferred into a 100 mL volumetric flask and added with distilled water to the mark line so the concentration of (0; 0.005; 0.01; 0.015; 0.02; 0.025 µg/mL) were obtained, and then the absorbance were measured by atomic absorption spectrophotometer at a wavelength of 193.7 nm. Absorbance versus

concentration of each solution was plotted to form calibration curve, then linearity of regression equation ($Y = aX + b$) and correlation coefficient were determined. From the measurement of the calibration curve, the line regression equation was $Y = 0.007655X + 0.001288$ and correlation coefficient (r) was 0.9998.

As much as 0.5 g of sample was weighed and then placed into a vessel, 5 mL of HNO_3 and 3 mL of HCl were added, then the solution was allowed to stand for 10 minutes to dissolve perfectly. The vessel was placed into the microwave at a temperature of 180°C for 30 minutes until the complete destruction obtained which was indicated by the formation of clear liquid. Then the digestion result were cooled and put into a 50 mL volumetric flask and distilled water was added up to 50 mL and filtered with Whatmann paper no.41. Then the absorbance of the solution was measured using atomic absorption spectrophotometer at a wavelength of 193.7 nm.

Calculation

Determination of the levels in the sample with the regression equation [14]:

$$Y = 0.007655X + 0.001288$$

$$\text{Concentration} \left(\frac{\mu\text{g}}{\text{g}} \right) = \frac{X \times V \times F_p}{W} \quad (1)$$

Note:

- Y = Absorbance
- X = Concentration of arsenic in the sample solution ($\mu\text{g/mL}$)
- V = Volume of sample solution (mL)
- Fp = Dilution factor (1)
- W = Sample weight (g)

Safety Index Assessment of Arsenic Levels in Rice

Based on [9] *Daily Intake* (DI) is expressed in milligrams of chemicals per kilogram of body weight (mg/kg) or referred to in ppm (*parts per million*). The amount of rice consumption used in this DI calculation used was 380 g, based on data obtained from the 2015 Central Statistics Agency, that the total rice consumption of Indonesian people is 380 g/day. The average adult's body weight is 60 kg.

Daily intake:

$$I = \frac{C \times R \times f_E \times D_t}{W_b \times t_{\text{Avg}}} \quad (2)$$

Notes :

- I = Intake (mg/kg/day)
- C = Arsenic concentration in rice (mg/kg)
- R = Total intake per day (kg/day)
- f_E = Annual exposure frequency (350 days/year)
- D_t = Duration of exposure, 30 years projection
- W_b = Body weight (kg)
- t_{Avg} = Average time period, 70 years x 365 days

After getting the daily intake value, excess cancer risk is calculated to find out whether the value is still safe to be consumed in a certain amount and time, using the formula:

$$\text{ECR} = I \times \text{CSF} \quad (3)$$

Notes:

- ECR = *Excess Cancer Risk*
- CSF = *Cancer Sloped Factor* (1,5)

3. Result and Discussion

3.1. Calibration Curves

The arsenic calibration curve was obtained by measuring the absorbance of the standard solution at a wavelength of 193.7 nm. In practice it is advisable to make at least four different standard concentrations and one blank to create a linear calibration curve that indicates the relationship between absorbance (A) and the concentration of analytes [12]. From the measurement of the calibration curve, the regression line equation was $Y = 0.007655X - 0.001288$. The calibration curve of standard solution of arsenic can be seen in Figure 1.

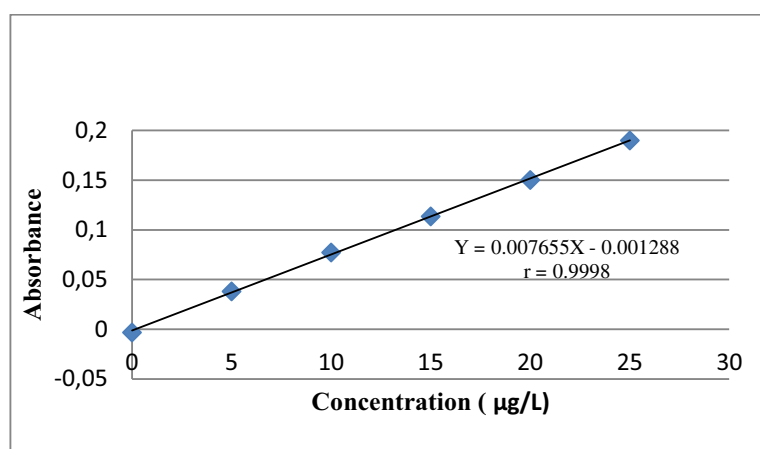


Figure 1. Calibration curve of standard arsenic solutions

Based on the Figure 1 there was linear relationship between concentration and absorbance, with the correlation coefficient (r) was 0.9998. This value $r \geq 0.9995$ indicated a linear correlation which stated the relationship between X (Concentration) and Y (Absorbance).

3.2. Arsenic Levels in Rice and Cooked Rice

The levels of arsenic in rice and cooked rice can be seen in Table 1 and Figure 2.

Table 1. Levels of arsenic in rice and cooked rice

No	Sample	Arsenic Levels (mg/Kg) (n=3)			Reduction in Arsenic Levels in Cooked rice (%)	
		Raw Rice	Cooked Rice with RO water	Cooked Rice with tap water	Cooked with RO water	Cooked with tap
1.	White rice	0.0693 ±0.0032	0.0541 ±0.0039	0.0614 ±0.0034	21.93	11.40
2.	Brown rice	0.0830 ±0.0040	0.0662 ±0.0139	0.0743 ±0.0072	20.24	10.48
3.	Red rice	0.3061 ±0.0096	0.2450 ±0.0043	0.2787 ±0.0131	19.96	8.95
4	Black rice	0.1091 ±0.0043	0.0865 ±0.0032	0.0984 ±0.0049	20.72	9.81

Note: Data is the means of three replications

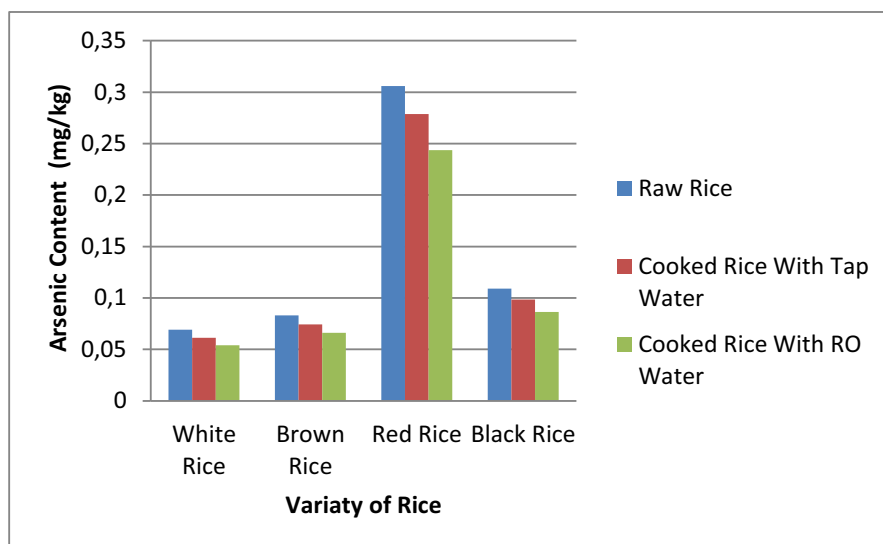


Figure2. Levels of arsenic in rice and cooked rice

Based on Table 1 and Figure 2 it was known that there was a difference in arsenic levels in rice and cooked rice. There are 4 types of rice tested: white rice, brown rice, red rice and black rice. The highest arsenic level of 0.3061 mg/kg was found in brown rice, and this level had exceeded standard (0.3 mg/kg) [9], while the lowest arsenic level of 0.0693 mg/kg was found in white rice. Similar results also reported by Ginting who found that in Indonesia the highest levels of arsenic was in brown rice (3.71 mg/kg) [15]. In addition, research conducted by the *Food Drug Administration* in 2012 to 2013 on 200 samples from all American regions found that the arsenic content in brown rice was higher than that of white rice. Many factors influence the level of arsenic in rice. If arsenic level in irrigation water is high, arsenic level in rice is also high, and vice versa. This is also closely related to genetic differences among the rice cultivar and the environment where rice is grown [7,16].

Arsenic level in white cooked rice using RO water decreased by 21.93% while when using tap water it decreased by 11.40%. In brown rice after being cooked with RO water arsenic level decreased by 20.24%, while when using tap water, the level decreased by 10.48%. In brown rice after being cooked using RO water, arsenic level decreased by 19.96%, while when using tap water, the level decreased by 8.95%. In cooked black sticky rice the arsenic level using RO water the arsenic level decreased by 20.75%, while after cooking with tap water, the arsenic content decreased by 9.81%.

Pal et al. reported that the cooking process was able to reduce arsenic level within the range of 8% - 58% [11]. Sengupta et al. also showed that cooking process was able to reduce arsenic level up to 28% when using different source of water (tap water, well water and rainwater) [12]. Similarly Halder et al. showed that the cooking process with tap water was able to reduce arsenic level within the range of 7.5% to 66.3% [13]. Parvanehvar showed the reduction of arsenic levels by 2.8% - 13.8% [17].

In this present study, it was able to be seen that the greatest decrease in arsenic level was after rice being cooked using RO water, which was 21.93 %. RO water does not contain minerals so that it is more active to absorb arsenic during cooking process. Silalahi and National Standardization Agency also set permitted maximum levels of arsenic in natural mineral water was 0.05 mg /L whereas in demineralized water was 0.01 mg/L [18,19].

3.3. Safety Index of Arsenic in Rice

The calculation of the safety of arsenic in rice based on WHO was obtained through the calculation of daily intake (*Daily Intake*) and then continued with *Excess Cancer Risk* (ECR) [5]. Safety index of arsenic in rice can be seen in Table 2.

Table 2. Safety index of arsenic in rice

No.	Sample	Arsenic	Daily Intake	ECR	ECR Safe
1	White rice	0.0541	1.8×10^{-4}	2.7×10^{-4}	Not safe
2	Brown rice	0.0662	2.2×10^{-4}	3.3×10^{-4}	Not safe
3	Red rice	0.3061	8.0×10^{-4}	12×10^{-4}	Not safe
4	Black sticky rice	0.1091	2.8×10^{-4}	4.2×10^{-4}	Not safe

Note: The safety index of arsenic in rice was calculated based on consumption rice in Indonesia that is 380 g/day/ person.

Based on data presented in Table 2, the values of ECR on white rice, brown rice, red rice, and black rice were greater than 1×10^{-5} , meaning that the rice was not safe when consumed at the amount of 380 kg for 350 day/year within 30 years by person with body weight of 60 kg or less. Chronic arsenic exposure can cause interference on biological systems such as digestion, respiratory, cardiovascular, endocrine, kidney, nerve, and reproduction, which eventually lead to increase cancer risk. Daily intake of 1 mg of arsenic can cause effects on the skin in a few years [20].

The high level of ECR was strongly influenced by the high consumption of rice in Indonesia, which was 380 g/day/person according to the Indonesian Statistics Agency in 2015. The higher the level of rice consumption, the higher the cancer risk. The standard of arsenic content in rice set by the WHO is 0.3 mg/kg based on the consumption of rice in America and Europe 9 - 50 g/day/person. In some Asian countries such as China and Japan, the majority of people still consume rice as a staple food, and the WHO standard is not used. The Japanese Food and Drug Supervisory Agency sets 0.01 mg/kg for arsenic levels in rice, while in China it is 0.15 mg/kg. In some Asian countries, especially in Indonesia, rice consumption is so high (200 - 400 g/day/person) that the people in Asian countries are more risky to arsenic exposure from rice [21-23].

4. Conclusions

The type of water used in cooking process significantly affected arsenic level in rice. The highest decrease in arsenic level when the rice was cooked using RO water was 21.93% and when using tap water was 11.40%. A daily supply obtained from the arsenic content in rice and the cooked rice (cooked using RO water and tap water) exceeded the safe limit set by WHO. It is suggested to the Indonesian Ministry of Health to regulate the permitted maximum of arsenic level in rice and their products.

References

- [1] Ismunandar 2007 *Popular Chemistry: From the Case of Mercury to the Sun's energy* ITB Bandung
- [2] Sambel D T 2015 *Environmental Toxicology* CV Andi Offset Yogyakarta 1: 13-115
- [3] WHO 2011 Joint FAO/WHO Expert Committee on Food Additives Evaluation of Certain Contaminants in Food: 51-52
- [4] Maekawa M 1998 Recent Information on Anthocyanin Pigmentation Rice Genetics *The Journal of Agriculture* 32 (13): 25-26
- [5] WHO 2011 Joint FAO/WHO Expert Committee on Food Additives Evaluation of Certain Contaminants in Food: 51-52.
- [6] Food Standards Agency 2007 Contract C101045: Arsenic in Rice Levels *Review Article*: 9-10

- [7] Duxbury J M, Mayer A B, Lauren J G, Hassan N 2003 Food Chain Aspects of Arsenic Contamination in Bangladesh: Effects on Quality and Productivity of Rice *Journal of Environmental Science and Health* 38 (1): 61-69
- [8] Das H K, Mitra A K, Sengupta P K, Hossain A, Islam F, Rabbani G H 2004 Arsenic Concentrations in Rice Vegetables and Fish in Bangladesh a Preliminary Study *Environmental International Journal* 30: 383-387
- [9] Food Drug Administration USA 2013 Center for Food Safety and Applied Nutrition *Arsenic in Rice and Rice Products in Risk Assessment Report 2012-2013* Version Released for Public Comment: 34-35
- [10] World Health Organization 2014 Codex Alimentarius Commission: *Codex Committee On Contaminants In Food*: Eight Session: 9
- [11] Pal A, Chowdhury U K, Mondal D, Das B, Nayak B, Ghosh A, Maity S and Chakraborti D 2009 Arsenic Burden from Cooked Rice in the Populations of Arsenic Affected and Non-Affected Areas and Kolkata City in West-Bengal India *Journal of Environmental Science and Health* 38 (1): 61-69
- [12] Sengupta M K, Hossain M A, Mukherjee A, Ahamed S, Das B, Nayak B 2006 Arsenic Burden of Cooked Rice: Traditional and Modern Methods *Journal of Food and Chemical Toxicology* 44: 1823- 1829
- [13] Halder D, Ashis B, Zdenka S, Debashis C, Jerome N, Gunnar Jet al 2014 Arsenic Species in Raw in Cooked Rice Implications for Human Health in Rural Bengal *Journal of Science of the Total Environment* 497 (10): 203
- [14] Gandjar I G and Rohman A 2007 *Analysis in Pharmaceutical Chemistry* Pusata Pelajar Yogyakarta 298 305-312,319
- [15] Ginting E E 2018 *Arsenic Analysis on Various Types of Rice Circulating in Medan City with Atomic Absorption Spectrophotometer* Thesis Faculty of Pharmacy University of North Sumatra Medan 58-60
- [16] Williams P N, Islam R M, Adomako E F, Raab A, Hossain U S A, Zhu G Y 2006 Increase in Rice Grain Arsenic for Regions of Bangladesh Irrigating Paddies with Elevated Arsenic in Ground Water *Journal of Environment Science Technology* 25 (10): 4093
- [17] Parvanehvar A 2015 *The Fate of Total Arsenic Content in Rice for Several Processing Variables Rinsing and High Volume Cook Water* Chicago: 36
- [18] Silalahi J 2014 Negative Impact of Reverse Osmosis (RO) Drinking Water on Health *J Indon Med Assoc.* 64 (5): 215-217
- [19] National Standardization Agency 2015 *Demineralized water* SNI 6241: 2
- [20] Smith E, Kempson I, Juhasz L A, Weber J, Skinner M W and Grafe M 2009 Localization of Speciation of Arsenic and Trace Element in Rice Tissue *Journal of Chemosphere* 76 (11): 536
- [21] Fu Y, Chen M, Bi X, He Y, Ren L, Xiang W 2011 Occurance of Arsenic in Brown Rice and ts Relationship to Soil Properties from Hainan China *Journal Environment Pollution* 159 (11): 1757-1762
- [22] Narukawa T, Hioki A, Chiba K 2012 Speciation and Monitoring Test for Inorganic Arsenic in White Rice *Journal Agriculture Food Chemistry* 58 (14): 813-818
- [23] Zhu G Y, Williams N P and Meharg 2008 Exposure to Inorganic Arsenic from Rice A Global Health Issue *Journal Environment Pollution* 154 (11): 159-161