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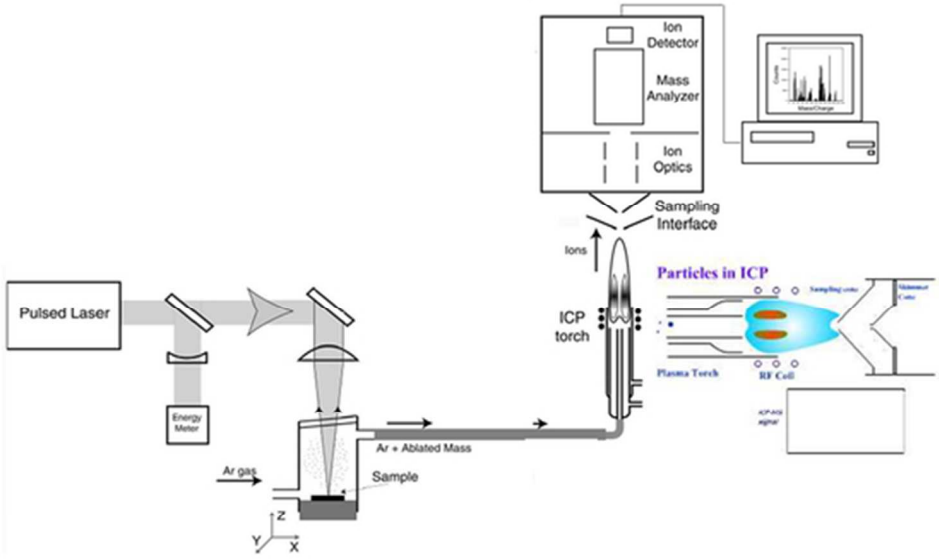

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Analysis of arsenic in rice grain using ICP-MS and fs LA-ICP-MS

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With rice being main staple crop in Asian countries such as China, Korea and Japan, the detection of arsenic (As), an element known to be carcinogenic to humans, has been the topic of high public interest. In this study, the total arsenic content in 200 white and 104 brown rice samples were collected in Korea and analyzed using Quadrupole Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). One of the rice grain sample was polished with 3, 5, 7, 9 and 11 degrees of milling and arsenic concentration variance from the surface to the inner core region was investigated. Furthermore, spatial distribution of arsenic over the cross section of a brown rice grain was obtained using Femtosecond Laser Ablation-Inductively Coupled Plasma- Mass Spectrometry (fs LA-ICP-MS). For the total arsenic content analysis, 91.7±28.1 and 101±33.6 ug/kg of arsenic was measured in the white and the brown rice respectively. The fs LA-ICP-MS mapping image explains the higher arsenic concentration in the brown rice is due to high arsenic distribution in the rice husk (protective covering of rice). Consequently, some degree of rice milling may be effective in the reduction of arsenic intake.

Introduction

Rice is an essential staple food crop in Asian countries such as China, Korea and Japan and accounts for as high as 35% of caloric and 20% of protein intake by a person[1]. The chemical composition of rice is known to be dependent on its varieties, milling ratio and storage period. While the brown rice consumption was not popular in the past due to its rough texture and lower digestibility, recent times saw a steady increase in its demand due to growing consumers' interest in healthier diet and new classes of brown rice with higher nutritional content and improved texture[2-6]. The rice grain is comprised of outer hull layers (pericarp, seed coat, and aleurone), embryo, and inner endosperm. By mass, the hull layers, embryo, and inner endosperm account for roughly 5-6%, 2-3%, and 91-93% of the total grain mass respectively[7, 8]. It has been reported that certain chemical constituents of rice are not evenly distributed over the entire grain but preferentially concentrated in the seed coat and aleurine layer[9]. Andrew et al. performed an imaging study for the surface of brown rice intentionally contaminated with arsenic using S-XRF and Bada et al. evaluated the feasibility of quantitative analysis of cadmium in rice flour using LA-ICP-MS[10,11].

Arsenic is an element classified as Category 1 carcinogen by Interenational Agency for Research on Cancer (IARC). The detection of arsenic in rice has raised a significant health concern on the safety of rice consumption around the world in the last 2~3 years[12, 13]. Toxic arsenic tends to accumulate in the human digestive track and in kidney when agricultural products cultivated or processed in contaminated water and soil is consumed[14]. Codex Alimentarius Commission does not provide any formal guideline on safe heavy metal content in either white or brown rice while China specifies safe content for arsenic to be below 0.2 mg/kg[15]. To date, there have been several studies focusing on understanding the content of toxic heavy metals and other nutritional elements for more popular white rice[16, 17]. However, such studies for the brown rice with different milling ratio have been rare.

This study seeks to investigate total arsenic content for both the white and brown rice with bulk digestion ICP-MS method and trace level arsenic distribution over a rice grain cross-section using LA-ICP-MS. Spatially resolved analysis by LA-ICP-MS may be useful in explaining the total arsenic content

difference between the white and brown rice and may provide useful analytical data for the field of agricultural food and environmental health.

Experimental

Instrumentation

The Quadrupole Inductively Coupled Plasma-Mass spectrometer (Nexion 300D, PerkinElmer, USA) was used to evaluate total arsenic content. Femtosecond laser ablation instrument (fs LA), (J200, Applied Spectra, Inc., USA) was coupled to the second ICP-MS (iCAP Q, Thermo, USA) to perform spatially resolved LA-ICP-MS analysis of arsenic. Table 1 summarizes experimental conditions for bulk digestion ICP-MS and fs LA-ICP-MS analysis.

1.0 L/min of Ar was used as a carrier gas for the bulk digestion ICP-MS. One of the main challenges in the analysis of the total arsenic content by bulk digestion ICP-MS is inaccuracy of the analysis caused by isobaric ion interference at m/z 75. The chloride interference is the prime example of such isobaric interference that must be addressed for the arsenic analysis. In the Ar plasma of ICP-MS, chloride can form $_{40}Ar_{35}Cl+$ that directly interferes at the same nominal mass-to-charge ratio as the As isotope. Some report that the chloride interference for the rice grain analysis may not be significant[18]. Nevertheless, the ICP-MS instrument in this study was operated under KED (Kinetic Energy Discrimination) mode in which He gas is injected to break up unwanted chloride species to eliminate the effect of interference[18].

For As distribution mapping over the rice grain by fs LA-ICP-MS, 5.76 mm^2 area was ablated with a 40 X 40 grid pattern with point spacing of 62.5 micron. The laser spot size was set at 60 microns.

Table 1. Optimized instrumental parameters for the analysis of rice samples by femtosecond laser ablation -inductively coupled plasma- mass spectrometry (fs LA-ICP-MS)

Laser ablation		Inductively Coupled Plasma Quadrupole Mass Spectrometry	
Wavelength	343 nm	R.F generator	Free-Running type, 40 MHz
Pulse duration	<480 fsec	R.F power	1400 W
Spot size	60 μm	Nebulizer gas flow rate	1.05 mL/min
Scan mode	Grid of spot	Interface cone	1×10^{-6} torr
Repetition rate	20 Hz	As/mass	75
Laser energy	120 μJ		
Total time	17600 s (5 h)		

Reagents and standards

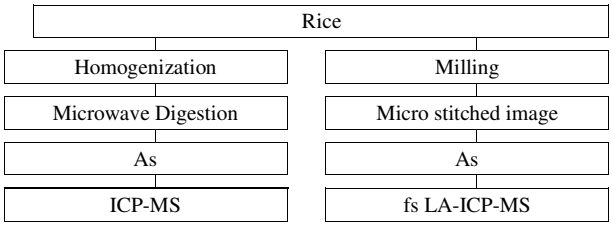
Reagents used for the analysis were certified reagents and deionized water was purified to 18.2 MΩ level using Milli-Q ultrapure water purification system (Millipore Co., MA, USA). Multi-element Calibration Standard 3 (PerkinElmer, USA) 10 mg/kg (in 5% HNO_3) was diluted to 10, 20, 50, 100, 150 ug/kg for ICP-MS instrument calibration. Semiconductor grade nitric acid (HNO_3, purity 70%, Dong Woo Fine Chem. Co. Ltd., Iksan, Korea) and hydrogen peroxide (H_2O_2, purity 30%, Dong Woo Fine Chem. Co. Ltd, Iksan, Korea) were used for digestion of test samples. Rice flour CRM from KRISS (Korea Research Institute of Standards and Science), (KRISS CRM 108-01-002) was used as calibration standards and also for quality control. Teflon vessel used for digesting rice samples was cleaned with distilled water, left overnight in 30% HNO_3, and then washed with deionized water prior to the use.

Samples preparation

For the total arsenic content analysis, 200 white rice samples (188 from South Korea, 6 from China, 3 from the US, 2 from Thailand, and 1 from Australia) and 104 brown rice samples (all 104 from South Korea) were collected. For assessing the impact of milling degree on the total arsenic content, one rice sample with relatively high total arsenic content was selected and polished to 3, 5, 7 and 11 degrees of milling using rice polishing machine (Barotec. Co. Ltd, Korea). For fs LA-ICP-MS arsenic imaging experiment, a cutting machine was used to cross-section a brown rice grain.

For sample preparation, the rice samples were dissolved in acid solution using Microwave digestion (ETHOS TOUCH CONTROL, Milestone, USA). 0.2 g of the rice sample was placed in the Teflon Vessel, 1 mL of H_2O_2 was subsequently added in the vessel and the sample was pre-treated at 60℃ for 10 minutes and at 80℃ for 25 minutes using heating block unit (ED16, LabTech, MA, USA). The pre-treated samples were then completely digested and dissolved by microwave digestion system after adding 2 mL distilled water. After the microwave digestion process, the solution was transferred and collected into 25 mL volumetric flask after cooling and washing of the vessel. The final prepared solution was filtered with a 0.45 μm syringe filter prior to the analysis by ICP-MS. Overall pre-treatment process of the sample are shown in Table 2.

Table 2. Analysis condition of sample preparation



Results and discussion

Method validation

In order to validate the analysis method, linearity, precision, accuracy, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were evaluated with CRM's. LOD was defined as three times the standard deviation of the 5 blank measurements and LOQ was similarly defined as 10 times the standard deviation of the 5 blank measurements[19]. LOD and LOQ values are estimated to be 0.087 and 0.290 μg / kg respectively. For the quantitative analysis of the total arsenic content, KRISS CRM was used as the certified materials of analysis and Table 3 summarizes the results. In order to ensure the reliability of analysis results, international quality control FAPAS (UK) test was performed. The Z-score of -0.1 meets the recommended requirement for $|Z| < 2$ to ensure high reliability of the analysis (Table 4).

Table 3. Analysis result of KRISS CRM (rice flour) for total arsenic .

Species	CRM Test			
	Ref. [a] (μg/kg)	Measure (μg/kg)	Recovery (%)	C.V (%)
1	2.02×10^3	2.21×10^3	109	
2	2.02×10^3	2.23×10^3	110	
3	2.02×10^3	2.21×10^3	109	
4	2.02×10^3	2.17×10^3	107	
5	2.02×10^3	2.09×10^3	103	
Total As	2.02×10^3	2.18×10^3	108	2.56

[a] Rice flour (KRISS CRM 108-01-002)

Table 4. Result of External quality control (FAPAS)

FAPAS	Chili powder	
Element	As	
Result	Satisfactory result|Z|<2	
	Z-score	As : -0.1

Analysis of total arsenic content in polished and brown rice

The total arsenic content in 200 white and 104 brown rice samples was shown in Table 5. The measured value was 91.7± 28.1 μg/kg and 101±33.6 μg/kg for the white and the brown rice samples respectively. The arsenic content was higher in brown rice than in polished rice. It is noted that there was a significant variability of the total arsenic content among different rice samples and this resulted in low precision of the measured value. This may be contributed to variability of milling process for different rice samples. Inconsistent thickness of husk and bran layer on top of white rice kernel may can lead to greater spread of the arsenic when milled layers contain arsenic.

According to the study by Pasias, the arsenic content in Greek white (or polished) rice was 197±43.0 μg/kg and in brown rice was 189±30.0 μg/kg[20]. These values are somewhat higher than what our current study suggests. Horner et al. reported the arsenic content in Californian white rice at 100± 10.0 μg/kg and in brown rice at 120±20.0 μg/kg[21]. On the other hand, Fu et al. reported the arsenic content in Vietnamese white rice to be 46.9~121 μg/kg and in brown rice to be 32.0~465 μg /kg[22]. These studies suggest that all rice contain a certain level of arsenic and its content is dependent on the geographical region. And the arsenic content is generally higher in the brown rice than in the polished rice, implying that arsenic may not be evenly spread over the entire rice grain but rather concentrated on external surfaces.

Table 5. Total arsenic in rice and brown rice

	Rice (μg/kg)	Brown rice (μg/kg)
Average	91.7±28.1[a]	101±34.6
Min	37.8	2.14
Max	194	234

[a] Average±stand deviation

Analysis of total arsenic content by milling ratio

Higher arsenic content is reported for the brown rice compared to the white rice. Additional experiments were performed to investigate the correlation of the total arsenic in the brown rice with respect to degree of rice milling. The arsenic content was found to be 169, 156, 136, 122 and 112 μg/kg for 3, 5, 7, 9 and 11 degrees of milling, respectively. With increasing milling ratio, the total arsenic content decreased (Figure 1). This trend also strengthens the argument that arsenic is not evenly distribute over entire rice grain volume and is likely to be concentrated on the external surface.

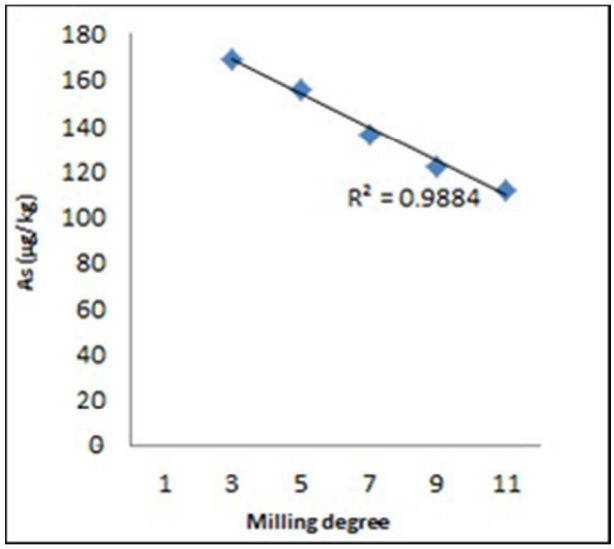


Figure 1. Correlation of arsenic content by milling of rice

Arsenic distribution imaging in brown rice

To confirm the above hypothesis generated from total As content measurement, the arsenic distribution imaging across entire section of the brown rice grain was investigated with fs LA-ICP-MS. From Figure 2, the mapping result clearly demonstrates that arsenic is present in significantly higher concentration on the external surface of the rice grain and its content decrease towards the inner core region.

Meharg et al investigated elemental distribution on the surface of the brown rice grain highly contaminated with arsenic (0.61 mg/kg) using S-XRF and LA-ICP-MS. His elemental imaging result shows only coarse arsenic distribution over the surface area and does not reveal arsenic content variation from the external surface to the inner core region of the rice grain[10]. In our current study, highly spatially resolved imaging result was obtained across the entire cross section of the brown rice grain using 1600 laser ablation spots. The brown rice samples in our study contained lower level arsenic content (~100 ppb per total arsenic content measurement). Since this is a bulk digestion result, the arsenic concentration in localized interior region of the rice grain can be lower than this average value. fs LA-ICP-MS has been researched for the analysis of various sample matrices and provides several analytical advantages. These advantages include smaller laser ablated particles for higher transport efficiency to ICP-MS, enhanced detection sensitivity, more homogeneous particle size distribution for higher precision performance and reduced matrix dependence of laser ablation[23,24]. fs LA-ICP-MS was chosen to ensure high sensitivity and precision for the arsenic detection based on very limited sampled mass with 20 laser pulses per location[25,26]. The technique provided adequate detection sensitivity to map arsenic content across the entire section of the brown rice grain with high spatial resolution.

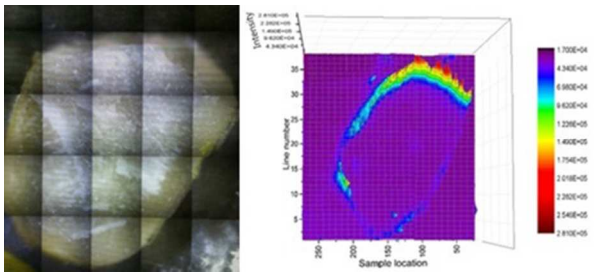


Figure 2. Arsenic intensity profile on a cross-section of a rice grain

Conclusions

Arsenic is a Category 1 carcinogen; IARC identifies arsenic and arsenic compound "carcinogenic to human". Arsenic is often absorbed into the body through human respiratory and digestive system. Therefore, detection of arsenic in rice has been a growing concern and topic of intense public interest, especially in Asian countries where the rice remains as a main staple crop.

Our current study along with past several other studies showed that the arsenic content in brown rice was higher than in polished white rice, which lead to the hypothesis of the preferential arsenic distribution near the surface region of the grain. Additional experiments in this study investigated the nature of arsenic distribution in the rice grains and these studies included measurement of total arsenic content in the rice samples with different milling degree and direct imaging of arsenic concentration over the grain cross section using fs LA-ICP-MS.

The arsenic content decreased with increasing milling ratio, signaling that arsenic is mainly distributed on near the surface rather than in the inner core of the grain. The direct arsenic concentration imaging with fs LA-ICP-MS clearly confirmed the arsenic concentration profile with significantly higher concentration near the surface of the grain. Our study confirmed ecological characteristic of arsenic distribution for the rice grains. Similar study focusing on nutritional microelements and harmful heavy metal elements would be beneficial to both ecological and environmental science community.

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Notes and references

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1 H. R. Kim, Y. H. Kwon, J. H. Kim and B. H. Ahn, *Korea Society of Food Science and Technology*, 2011, **43**, 142-148.
2 S. K. Oh, J. H. Lee, M. R. Yoon, D. J. Kim, D. H. Lee, I. S. Choi, J. S. Lee, I. H. Kim and J. S. Lee, *Journal of the Korean Society of Food Science and Nutrition*, 2012, **41**, 963-969.
3 K. H. Mo, S. G. Choi and J. S. Lee, *J Agric Life Sci*, 2006, **40**, 41-48.
4 S. L. Kim, Y. K. Son, J. R. Son and H. S. Hur, *Korean J Crop Sci*, 2001, **46**, 221-228.
5 S. S. Kim, Y. K. Kim and W. J. Lee, *Korean J Food sci Technol*, 1998, **30**, 1107-1113.
6 Y. S. Oh, *Study on nutritional properties of sprouting brown rice MS thesis Kongju Nation University Kongju Korea*, 2002.
7 K. Hoshikawa, The growing rice plant : An anatomical monograph Noubunkyo Tokyo, 1989, 225-292.

8 RDA, Rural Development Administration.
9 J. S. Choe, H. H. Ahn and H. J. Nam, *J. Korean Soc. Food Sci. Nutr*, 2002, **31**, 885-892.
10 A. A. Meharg, E. Lombi, P. N. Williams, K. G. Scheckel, J. Feldmann, A. Raab, Y. G. Zhu and R. Islam, *Environ Sci Technol*, 2008, **42**, 1051-1057.
11 K. Baba, E. Watanabe, H. Eun and M. Ishizaka, *J Anal Atom Spectrom*, 2003, **18**, 1485-1488. DOI: Doi 10.1039/B310827a.
12 *AGENTS CLASSIFIED BY THE IARC MONOGRAPHS, VOLUMES*, 2013, **1-109.**
13 ConsumerReports, *Arsenic in your food Our findings show a real need for federal standards for this toxin, http://www.consumerreports.org/cro/magazine/2012/11/arsenic-in-your-food/index.htm*, 2012.
14 H. K. Das, A. K. Mitra, P. K. Sengupta, A. Hossain, F. Islam and G. H. Rabbani, *Environ Int*, 2004, **30**, 383-387.
15 *CODEX GENERAL STANDARD FOR CONTAMINANTS AND TOXINS IN FOOD AND FEED, CODEX STANDARD*, 2012.
16 J. H. Moon, M. Ebihara, B. F. Ni, B. Arporn, P. Setyo, R. M. Theresia, B. S. Wee, N. A. Abd. Salim, P. C. B.Pabroa, J Radio anal Nucl Chem, 2012, **291**, 217-221.
17 W H. Lamont, Journal of Food Composition and Analysis, 2003, **16**, 687–695.
18 KFDA, Risk Assessment of Heavy Metals in Infant & Young Children Foods, *Korean Food and Drug Administration, Cheongwon, Korea.*, 2012.
19 KFDA, *Korean Food Standards Codex. Korean Food and Drug Administration, Cheongwon, Korea.*, 2012, **4**, 1-5.
20 I. N. Pasias, N. S. Thomaidis and E. A. Piperaki, *Microchem J*, 2013, **108**, 1-6.
21 N. S. Horner and D. Beauchemin, *Analytica chimica acta*, 2013, **758**, 28-35.
22 J. Fu, Q. Zhou, J. Liu, W. Liu, T. Wang, Q. Zhang and G. Jiang, *Chemosphere*, 2008, **71**, 1269-1275.
23 F. Poitrasson, X. Mao, S. S. Mao, R. Freydier and R. E. Russo, *Anal Chem*, 2003, **75**, 6184-6190.
24 R. E. Russo, X. L. Mao, J. J. Gonzalez and S. S. Mao, *J Anal Atom Spectrom*, 2002, **17**, 1072-1075.
25 J. J. Gonzlez, D. Oropeza, X. Mao and R. E. Russo, *J Anal Atom Spectrom*, 2007,**23**, 229-234.
26 J. J. Gonzalez, C. Liu, S. B. Wen, X. Mao and R. E. Russo, *Talanta*, 2007, **73**, 577-582.