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Revealing the Spatial Distribution of Inorganic Elements in Rice Grains

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Femtosecond laser ablation (fs LA) was used in this study to identify pollution by heavy metals and the distribution of elemental nutrients at different rice milling ratios. Polished rice (degrees of milling of 3, 5, 7, 9, and 11) was collected from major Korean supermarkets and one sample thereof was selected. An internal quality control experiment was conducted using a rice flour certified reference material from the Korea Research Institute of Standards and Science (KRISS CRM) for the evaluation of the efficacy. To assess the effectiveness of the analysis method, the reliability was validated using a food analysis performance assessment scheme (FAPAS), with chili powder serving as an external quality control. The results of the analysis of the inorganic elements Ti, Ca, Al, Fe and Mn in white and brown rice with degrees of milling of 3, 5, 7, 9 and 11 using ICP-MS, ICP-OES and AAS revealed contents of 0.40, 49.2, 2.43, 5.36 and 10.3 mg/kg in white rice and 0.59, 78.0, 7.52, 11.0 and 18.5 mg/kg in brown rice, respectively. Among the elements, there were remarkable differences in the measured contents. By comparing the contents of the elements at different degrees of milling, Ti, Co, As, Ca, Al, Cu, Fe, and Mn were determined to be distributed on the surface of the rice grains, whereas the contents of Cd and Pb increased toward the center of the rice grains, and Si was evenly distributed. After the quantitative analysis of rice samples polished to different degrees of milling, Ca and Al, which were contained in large amounts, and Si were analyzed with specificity by fs LA. The results show that Ca and Al were distributed in the rice husk (protective covering of rice) and Si was distributed in all parts of the rice.

Key Words : fs LA, ICP-OES, AAS, ICP-MS, Rice

Introduction

Rice is a staple food in East Asia, including in Korea and Japan.[1] Rice has a significant effect on the national health of these countries because its consumption accounts for approximately 35% of the caloric intake and approximately 20% of the protein intake per person.[2] In developing countries, the amount of rice consumed accounts for 715 kcal/capita/day and is the source of 27% of dietary energy, 20% of dietary protein, and 3% of dietary fat. In Guinea, Gambia, Senegal, and Cote d'Ivoire, rice supplies 22–35% of dietary energy and 23–34% of dietary protein. In Bangladesh, Laos, Vietnam, Myanmar and Cambodia, rice supplies > 50% of dietary energy and protein and 17–27% of dietary fat.[3] The inorganic nutrient components of rice are affected by the variety of rice, the milling ratio and the storage period.[4] Polished rice is produced by removing the husk and the rice bran through a milling process, and brown rice is produced through a milling process that includes the husk.[5] The degree of milling indicates the extent to which the rice grain, composed of the outer hull layers (pericarp, seed coat, and aleurone), is polished. Additionally, white rice only contains starch due to the removal of the outer hull layers. The extent of rice milling is described by the milled ratio or the degree of milling. Brown rice is composed of 5–6% rice bran, 2–3% germ and 92% endosperm. The theoretical milled ratio obtained when the rice bran and germ are removed is 91.2%, which is defined as a milling degree of 11. That is, a degree of milling of 11 describes polished rice in which the bran layer and germ have been completely removed, and milling degrees of 3, 5, and 7 describe brown rice in which 2%, 4% and 6%, respectively, of the bran layer has been removed.[6] The demand for brown rice has been increasing because the latest health trend has aroused consumer interest in health foods. Thus, functional brown rice with strengthened trophism and functionality as well as improved texture and appeal has been developed. However, the consumption of brown rice has not been high because of its rough texture and low digestibility.[7,8] Recent attention directed toward the distribution of trace elemental nutrients in staple crops has included a focus on the presence of trace elements in rice grains.[9] The constituents of brown rice are not uniform over the entire grain but are contained in the areas of the endosperm and the germ composed of the rind, the testa, and the aleurone layer. The milling degree is a significant factor affecting the elemental nutrients found in rice grains.[10]

The contents of certain elements and compounds, including fats, proteins, iron, calcium, and thiamine, are proportional to the milling degree, and when the degree of milling is increased to improve the taste of cooked rice, a proportional loss of many of these elements occurs.[11-13]

Previous research has shown that Cu, Fe, Mn and Zn are mainly found on the surface of the rice grain, whereas Cd and Ni are found inside the grain.[14]

No standard specifications for the heavy metal content of polished rice or brown rice has been established in the Codex Alimentatarius Commission (Codex). The standard specification for the Cd content in polished rice is 0.4 mg/kg in Korea. In the case of brown rice, the standard specification for the Cd content is 0.4 mg/kg in Japan, and the standard specifications for Cd and Hg are 0.2 and 0.02 mg/kg, respectively, in China.[15]

Although studies on the distribution of nutrients or heavy metals have generally been conducted on polished rice, no significant study on the effect of milling on the nutritional content of brown rice has been carried out.[16,17] This study was conducted to contribute to national well-being by identifying the heavy metal contents and the distribution of inorganic nutrients in rice grains with milling degrees of 3, 5, 7 and 11. The rice samples were examined using femtosecond laser ablation (fs LA), and quantitative analysis was conducted using inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES) and atomic absorption spectrophotometry (AAS).

Experimental

Sample Collection. Rice milled to milling degrees of 3, 5, 7 and 11 using a rice swipper (Barotec Co. Ltd, Korea) was used for this experiment after randomly selecting a sample from a major Korean supermarket. The cross-section of the rice grain used for fs LA imaging was analyzed after cutting the grain in half widthwise (Figure 1).

Analysis. To analyze the inorganic elements, 2 g of polished rice (milling degrees of 3, 5, 7, and 11) was weighed in a Teflon beaker and then dissolved at 200 °C using HNO_3 (approximately 5 mL). The solution was cooled to room temperature and then adjusted to a constant volume in a 25 mL volumetric flask with DI water. A fs LA instrument (J200 Applied Spectra, Inc., Fremont, CA, USA) was used for image analysis of the inorganic elements (Ti, Cd, Pb, Ca, Si, Fe, and Mn). ICP-MS (iCAP Q, Thermo Scientific Inc., Waltham, MA, USA) was used for the quantitative analysis of Ti, Co, Cd, and Pb. ICP-OES (iCAP 6500 Duo, Thermo Scientific Inc., Waltham, MA, USA) was used for the quantitative analysis of Ca, Si, and Al. AAS (Ice 3000 Series, Thermo Scientific Inc., Waltham, MA, USA) was used for the quantitative analysis of Cu, Fe, and Mn. Table 1 shows the operating conditions for the ICP-MS, ICP-OES, AAS, and fs LA experiments. To determine the distribution of the inorganic elements in a rice grain, point spacing was performed to 68.5 μm over an area of 5.76 mm^2 using a 28 × 30 grid pattern, and the laser spot diameter was 60 μm. The analysis was conducted with He at a flow rate of 0.2 mL/min as the carrier gas in fs LA and with Ar at a flow rate of 1.0 mL/min as the carrier gas in ICP-MS.

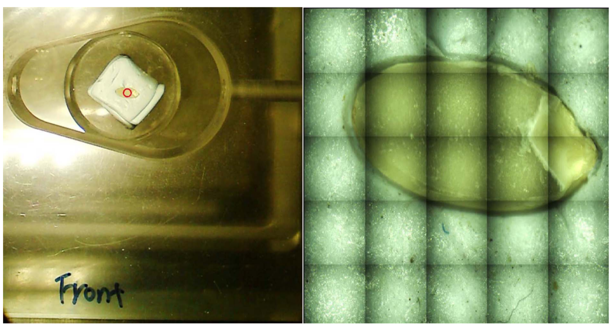

Figure 1. Cross-section of a rice grain after cutting it in half widthwise.

Table 1. Operating conditions of LA-ICP-MS, AAS and ICP-OES

Laser ablation		Inductively coupled plasma mass spectrometry	
Pulse duration	< 480 fs	R. F generator	Free-Running type, 40 MHz
Pulse width	3 μs	R. F power	1400 W
Spot diameter	60 μm	Nebulizer gas flow rate	1.05 mL/min
Scan mode	Grid of spot	Interface cone	1×10^{-6} Torr
Repetition rate	40 Hz	Pb, Cd, Ti, Co/mass	208, 112, 48, 59
Laser energy	120 μJ		
Fluence	4.25×10^2 mJ		
Total time	17600 s (5 h)		
Ca,	422.6,		
Al,	394.4,		
Si/nm	390.5		
Atomic absorption spectrometry		**Inductively coupled plasma optical emission spectrometry**	
Power	50 or 60 Hz	Plasma	1150W
Measurement mode	Absorbance	Nebulizer gas flow rate	0.70 mL/min
Bandpass	0.5 nm	Auxiliary gas flow	0.5 L/min
Fuel flow	1.1 L/min	Nebulizer	1 L/min
Nebulizer uptake	4 sec	Spray chamber	Cyclonic type
Flame type	Air-C_2H_2	Torch	EMT Duo
Mn,	279.5,	Ca,	373.6,
Cu,	324.8,	Si,	251.6,
Co/nm	248.8	Al/nm	396.1

Reagents and Standards. All of the reagents used for the analysis conducted in this study were guaranteed reagents. Deionized water (> 18.2 MΩ cm) provided by a Milli-Q ultrapure water purification system (Millipore Co., Billerica, MA, USA) was used in all experiments. Stock solutions (10 mg/kg) were prepared as working standards for the analysis of Ti, Cd, Pb, and Mn (PerkinElmer Inc., Waltham, MA, USA), and 1000 mg/kg stock solutions were prepared for the analysis of Ca, Si, Al, Cu, and Fe (PerkinElmer Inc., Waltham, MA, USA). Nitric acid (70%, electronic grade, Dong Woo

Table 2. Results obtained for three analyzed certified reference materials (n=7)

Element	SD (μg/kg)	LOD (μg/kg)	LOQ (μg/kg)
Pb	0.014	0.053	0.18
Cd	0.007	0.025	0.080
Al	0.241	2.45	8.18

Fine Chem Co. Ltd., Iksan, Korea) was used for sample dissolution. Rice flour (108-01-002, Korea Research Institute of Standards and Science (KRISS), Daejeon, Korea) was used as a certified reference material (CRM) for quality control. All glassware was dipped in 30% HNO_3 overnight and then washed with distilled water before use.

Results and Discussion

Method Validation. The limits of detection (LODs) and

Table 3. Analysis results of KRISS CRM (rice flour) for Cd and Pb

Element	Rice flour (fortified) KRISS CRM 108-01-002			
	Certified value (mg/kg)	Determined value (mg/kg)	RSD (%)	Recovery (%)
Cd	1.32	1.20	3.60	90.91
Pb	1.18	1.12	5.23	94.92

Table 4. Results of external quality control (FAPAS)

FAPAS	07196 Chili powder	
Element	Pb	Cd
Result Z-score Satisfactory result |Z| < 2	0.2	−0.2

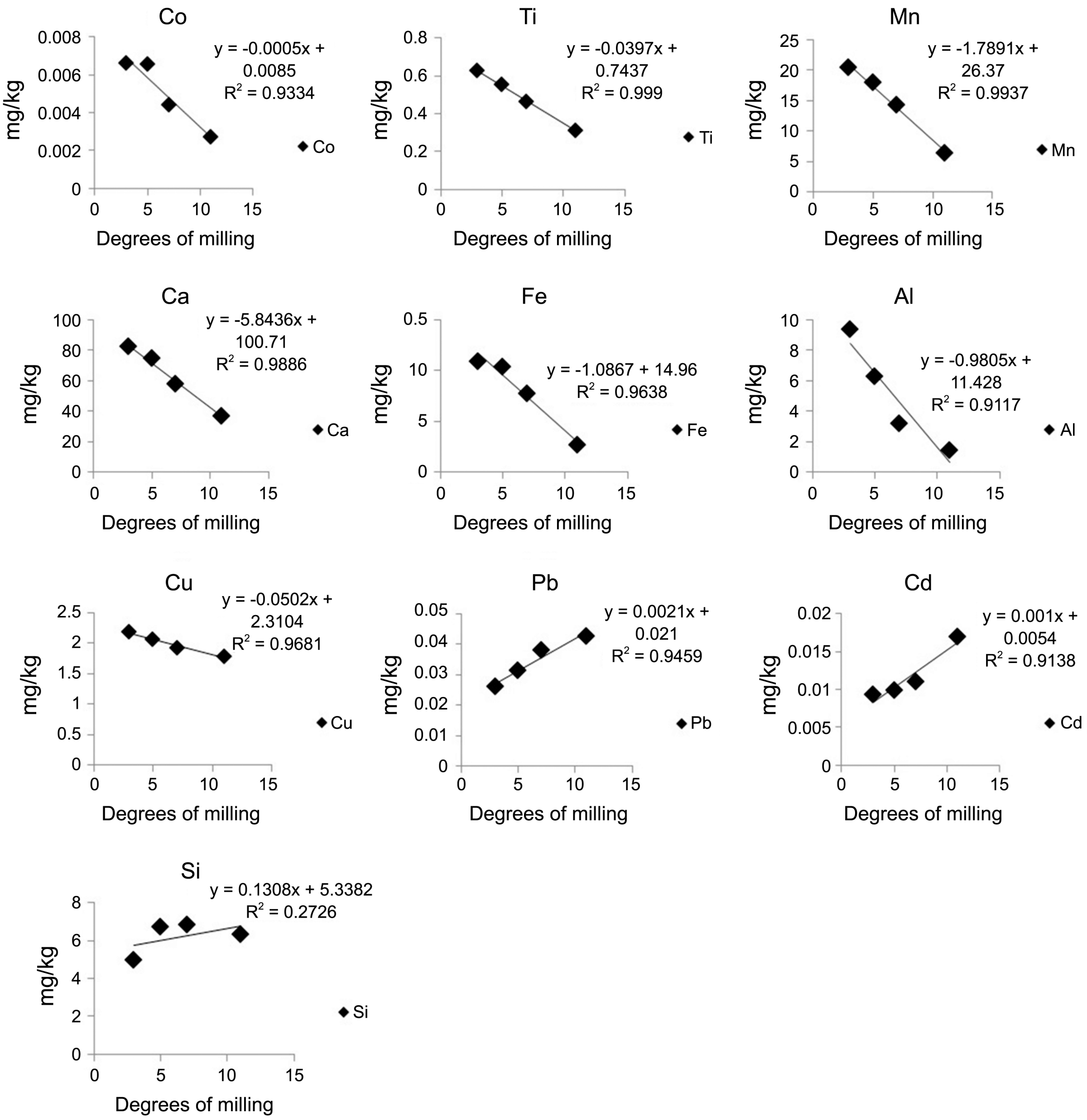

Figure 2. Correlation of the inorganic elemental contents with the milling ratio of rice.

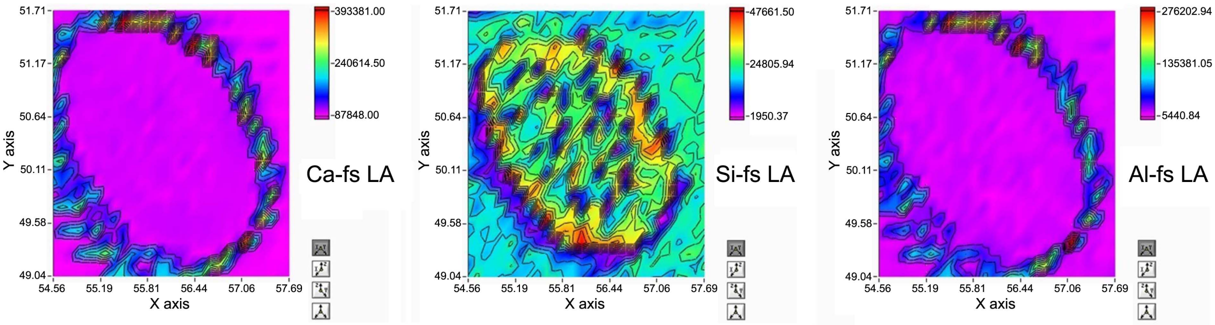

Figure 3. fs LA Ca, Al, and Si intensity profiles over a cross-section of a rice grain. The color bar in the upper left corner indicates that the abundance of an element increases from purple to red. Two hundred shots were performed per spot using a laser speed of 0.05 mm/s for fs LA analysis.

the limits of quantification (LOQs) were determined using reference values of 3 × standard deviation (SD) and 10 × SD, respectively, based on the guidelines for the monitoring of harmful substances of the Korean Food and Drug Administration (KFDA).[18] The LODs, LOQs, and SDs were 0.025–2.45, 0.080–8.18, and 0.007–0.24 µg/kg, respectively (Table 2). The reliability of the analysis results for heavy metals was ensured by using an internal quality control and a food analysis performance assessment scheme (FAPAS), an international quality control method. For the internal quality control, the relative SDs (RSDs) and recovery rates were 3.60–5.23% and 90.91–100.50%, respectively. Z-scores, which are a measure of reliability, were calculated to be 0.2, and −0.2, for Pb, and Cd, respectively, which were both within the acceptable range of $|Z| < 2$.[19] (Tables 3, 4).

Quantitative Analysis of the Inorganic Elements in Brown Rice and Polished Rice. The contents of Ti, Co, Cd, Pb, Ca, Si, Al, Cu, Fe, and Mn were determined to be 0.40, 0.0045, 0.013, 0.034, 49.2, 5.15, 2.43, 1.77, 5.36, and 10.3 mg/kg, respectively, for polished rice, and 0.59, 0.0063, 0.13, 0.010, 0.028, 78.0, 5.51, 7.52, 2.25, 11.0, and 18.5 mg/kg, respectively, for brown rice. All of the elements except Cd and Pb were detected at higher levels in brown rice than in polished rice.

In the study by Horner *et al.*, the contents of Cu, and Fe in polished rice were determined to be 0.17 ± 0.009 and 0.50 ± 0.20 mg/kg, respectively, and the contents of Cu, and Fe in brown rice were determined to be 0.40 ± 0.10 and 1.10 ± 0.40 mg/kg, respectively.[20] The results of this study agree with those of Honer *et al.*, and Cu and Fe are normally contained in higher concentrations in brown rice than in white rice. The contents of inorganic elements are generally higher in brown rice than in polished rice, meaning that Ti, Co, Ca, Al, Cu, Fe, and Mn are concentrated on the external surface of the rice grain. In addition, Cd and Pb, which are known to be heavy metals harmful to humans, were detected at higher levels in the polished rice than in the brown rice, indicating that they are concentrated inside the rice grain.

Analysis the Inorganic Elements by Degree of Milling. Experiments were performed to determine the correlation between the levels of trace elements and the milling ratio. As shown in Figure 2, the highest contents of Ti, Co, Ca, Al, Cu, Fe, and Mn were detected for a milling degree of 3 at levels of 0.62, 0.01, 81.1, 8.60, 2.29, 10.9, and 19.7 mg/kg, respectively. The highest contents of Cd and Pb were detected for a milling degree of 11 at levels of 0.015 and 0.037 mg/kg, respectively. As the milling ratio was increased, the contents of Ti, Co, Ca, Al, Cu, Fe and Mn decreased, indicating that these elements are mainly distributed on the external surface of the rice grain; on the other hand, Pb and Cd are distributed in the interior of the rice grain, and Si is relatively evenly distributed.

Distribution of the Trace Elements by Degree of Milling. Rice contains several inorganic elements, including harmful heavy metals, as well as beneficial substances. To determine the distribution of inorganic elements in brown rice and polished rice, the distribution of inorganic elements in rice grains was analyzed using fs LA. As shown in Figure 3, Ca and Al were mainly distributed on the external surface of the rice grain and on the cereal grain, and the contents of these elements decreased moving toward the inside of the rice grain. Si was distributed in several areas of the rice grain. The results of this experiment are similar to those of Meharg *et al.*, studied the distribution of elements on the surface of contaminated brown rice using synchrotron X-ray fluorescence (S-XRF) and LA-ICP-MS.[14]

Comparing our research concerning the quantitative analysis of inorganic elements with that of Meharg *et al.*, it could be deduced that Mn and Cu are concentrated in the outer hull layers of rice grains. In the study by Meharg *et al.*, imaging data for inorganic elements were obtained from the surface of brown rice samples using S-XRF, but the distribution of As was determined based on the signals gathered from 39 spots using LA-ICP-MS, which has a low resolving power and sensitivity. In this study, differentiated results were obtained by well-resolved signals from the analysis of an 840 pixel cross-section of a rice grain using fs LA. A comparison of the fs LA results with those obtained using the types of equipment employed to analyze liquid samples reveals many advantages in using fs LA to analyze solid samples directly. Among the advantages are the use of a minimum amount of sample, no poisonous waste, depth profiling, no sample preparation and no exposure to harmful substances.[21-23]

Conclusion

This study investigated how the contents of inorganic elements in rice were affected by the milling ratio. Quantitative analysis was conducted using ICP-MS, ICP-OES, and AAS, and the distribution of the investigated inorganic elements and heavy metals in a rice grain was imaged using fs LA. Brown rice was observed to contain more Ti, Co, Ca, Al, Cu, Fe, and Mn than polished rice, whereas Pb and Cd were detected at higher levels in polished rice. The relationship between the contents of the inorganic elements in rice and the milling ratio was confirmed. The contents of Ti, Co, Ca, Al, Cu, Fe, and Mn decreased as the milling ratio increased, whereas the contents of Cd and Pb increased as the milling ratio increased. To confirm this distribution of the elements, experiments were carried out using fs LA to image the distribution of inorganic elements in polished and brown rice. This experiment confirmed that Ca and Al were distributed on the external surface of rice grain and that the contents of these elements decreased moving toward the interior of the rice grain, whereas Si was observed to be evenly distributed in the rice grain.

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References

1. Armienta, E. S. In: *Proceedings of the VIII International Rice Conference for Latin America and the Caribbean*, Cuevas-Perez, F., Ed.; Villahermosa, Tabasco, Mexico, 10-16 November 1991; p 5-8.
2. Kim, H. R.; Kwon, Y. H.; Kim, J. H.; Ahn, B. H. *Korea Society of Food Science and Technology* **2011**, *43*, 142.
3. FAOSTAT Retrieved 2 May 2001 from the World Wide Web: http://apps.fao.org/. 2001.
4. Conway, J. A., Sidik, M., Halid, H. *Proceedings of the Fourteenth ASEAN Seminar on Grain Postharvest Technology*; Manila, Philippines, 1991; pp 55-82.
5. Ha, T. Y.; Ko, S. N.; Lee, S. M.; Kim, H. R.; Chung, S. H.; Kim, S. R.; Yoon, H. H.; Kim, I. H. *Eur. J. Lipid Sci. Tech.* **2006**, *108*(3), 175.
6. Hoshikawa, K. *The Growing Rice Plant: An Anatomical Monograph*; Noubunkyo: Tokyo, 1989; pp 225-292.
7. Juliano, B. O. *Rice: Chemistry and Technology*, 2nd ed.; American Association of Cereal Chemists: St. Paul, Minn., USA, 1985.
8. Kim, S. L.; Son, Y. K.; Son, J. R.; Hur, H. S. *Korean J. Crop Sci.* **2001**, *46*, 221.
9. UNICEF *UNICEF South Asia Regional Workplan* 1998 *Programme Priorities and Regional Tasks*; UNICEF Regional Office, 1998.
10. Champagne, E. T.; Marshall, W. E.; Goynes, W. R. *Cereal Chem.* **1990**, *67*(6), 570.
11. Park, J. K.; Kim, S. S.; Kim, K. O. *Cereal Chem.* **2001**, *78*(2), 151.
12. Mcgaughe, Wh. *Journal of Economic Entomology* **1970**, *63*(4), 1375.
13. Sun, H.; Siebenmorgen, T. J. *Cereal Chem.* **1993**, *70*(6), 727.
14. Meharg, A. A.; Lombi, E.; Williams, P. N.; Scheckel, K. G.; Feldmann, J.; Raab, A.; Zhu, Y. G.; Islam, R. *Environ. Sci. Technol.* **2008**, *42*(4), 1051.
15. *Codex General Standard for Contaminants and Toxins in Food and Feed*, Codex Standard 2012.
16. Liang, H.; Li, Z.; Tsuji, K.; Nakano, K.; Nout, M. R.; Hamer, R. J. *J. Cereal Sci.* **2008**, *48*(1), 83.
17. Liu, K. L.; Cao, X. H.; Bai, Q. Y.; Wen, H. B.; Gu, Z. X. *J. Food Eng.* **2009**, *94*(1), 69.
18. KFDA *Korean Food Standards Codex. Korean Food and Drug Administration*, Cheongwon, Korea, 2012; Vol. 4, p 1.
19. FAPAS *Food Analysis Performance Assessment Scheme. Proficiency Testing Report 07196*, August-September 2013, The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK., 2013.
20. Horner, N. S.; Beauchemin, D. *Analytica Chimica Acta* **2013**, *758*, 28.
21. Bleiner, D.; Lienemann, P.; Vonmont, H. *Talanta* **2005**, *65*, 1286.
22. Guillong, M.; Gunther, D. *J. Anal. At. Spectrom.* **2002**, 17(*8*), 831.
23. Kuhn, H. R.; Gunther, D. *J. Anal. At. Spectrom*. **2004**, *19*, 1158.