

Research Article



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## HPLC-ICP-MS를 활용한 잡곡의 비소 화학종 및 위해 분석

안재민, 홍경숙, 김성연, 김대중, 이호진, 신희창\*

### Arsenic Speciation and Risk Assessment of Miscellaneous Cereals by HPLC-ICP-MS

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#### Abstract

**BACKGROUND:** Miscellaneous cereal have been largely consumed in Korea as due to their physiological functions beneficial to human health. The cereals are currently a social concern because they have been found to contain heavy metals. Thus, monitoring heavy metals in the cereals is an important requirement for food safety analysis. In this study, we determined arsenic concentration in the cereals randomly harvested from different markets.

**METHODS AND RESULTS:** Inorganic arsenic was determined by ICP-MS coupled with HPLC system. The HPLC-ICP-MS analysis was optimized based on the limit of detection and recover test to reach 0.13-1.24 µg/kg and 94.3-102.1%, respectively.

The concentrations of inorganic arsenic equivalent to daily exposure were levels of 19.91 µg/day in mixed grain, 1.07 µg/day in glutinous rice, 0.77 µg/day in black brown rice, 0.13 µg/day in barley and 0.11 µg/day in soybeans.

**CONCLUSION:** The levels of arsenic in miscellaneous cereals were found lower than the recommended The Joint FAO/WHO Expert Committee on Food Additives (JECFA)

levels, suggesting that the cereals marketed in Korea are not potential concern in risk assessment.

**Key words:** Arsenic species, Miscellaneous cereals, PTWI, Risk assessment

#### 서론

잡곡은 다양한 영양소와 기능성 성분을 함유하고 있어 건강에 유익한 것으로 알려져 있다. 그러나 최근 몇몇 잡곡에서 중금속, 특히 비소(arsenic)의 검출이 보고되면서 소비자들의 우려가 커지고 있다. 비소는 인체에 유해한 중금속 중 하나로, 장기간 섭취하면 암, 신경장애, 혈액장애 등을 유발할 수 있다. 따라서 잡곡의 비소 농도를 정확히 측정하고, 그 위험성을 평가하는 것은 매우 중요하다. 본 연구는 HPLC-ICP-MS를 이용하여 다양한 잡곡에서 비소의 화학종(비소화합물)을 분석하고, 이를 바탕으로 인체에 대한 위해도를 평가하였다. 분석 결과, 조사된 잡곡의 비소 농도는 대부분 WHO 권고량 이하로 나타났다. 이는 국내에서 유통되는 잡곡의 비소 위험도가 낮을 것으로 판단된다. 그러나 지속적인 모니터링과 안전 관리가 필요하다. (Devesa, 2001). Kim (2000)

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(arsenic species) As(III)>As(V)>MMA>DMA>AsC>AsB

가  
(International Agency for Research on Cancer, IARC)  
가

Group 1 (carcinogenic to humans)  
(IARC, 2004). JECFA

(PTWI, Provisional Tolerable Weekly Intake) 0.015 mg/kg b.w./week 1988  
가 (JECFA, 1988).

2010 JECFA  
가 PTWI  
PTWI

(JECFA, 2010; JECFA/72/SC, 2010).  
가

( )  
가 가  
(Munoz et al., 2002).

가  
CODEX (CODEX, 2012).

가  
가 가

**재료 및 방법**

**실험재료**

2015-2016  
188  
가

( 2015-52 )  
1-3 kg

**Table 1. Sampling items of miscellaneous cereals in Korean markets**

Sample	Total
black rice	14
oat	14
millet	14
mung bean	13
barley	16
sorghum	18
adlay	14
foxtail millet	16
glutinous rice	12
soy bean	16
adzuki bean	16
mixed grain	25
Total	188

Table 1

**시약 및 초자**

70%  
(Dong woo Fine-Chem, Korea) 30% (Dong woo Fine-Chem, Korea)

Milli-Q ultrapure water purification system (Millipore Co., Massachusetts, USA) 18.2 MΩ

3 (P.E.) , 5%

24 3  
1,000 mg/L (Merck, Germany) 5%

(CRM, Certified Reference Materials) National Institute of Standards and Technology (NIST) 1568b (Rice Flour, USA) NMJJ 7503-a (Rice Flour, Japan)

arsenobetaine (Fluka, Switzerland), dimethylarsinic acid (Chem Service, USA), disodium methyl arsenate (Chem Service, USA), arsenite (High-Purity Standards, USA), arsenate (High-Purity Standards, USA) 1,000 mg/L stock solution, 10 mg/L 1%

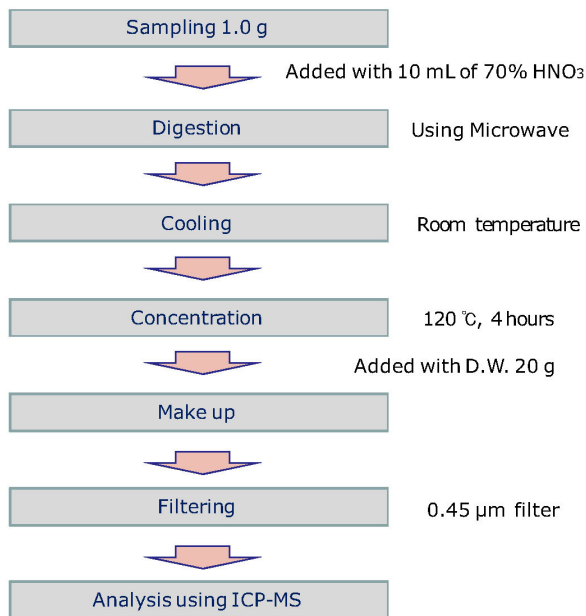
working solution  
standard solution

HPLC  
ammonium carbonate(Fluka, Switzerland), ammonium phosphate(Fluka, Switzerland)

**Table 2. Operating condition of microwave instrument**

Step	Power (Watt)	Time <sup>a)</sup> (min)	Temp <sup>b)</sup> (°C)	Hold <sup>c)</sup> (min)
1	1600	5:00	90	10:00
2	1600	5:00	150	10:00
3	1600	5:00	190	30:00

<sup>a)</sup>Ramping time, <sup>b)</sup>Target temperature, <sup>c)</sup>Stay time in target temperature



**Fig. 1. Flow chart for total arsenic analysis.**

1% 가

**시료의 전처리**

7. 가 (Wang *et al*, 1991).  
 7.1. 가 (Inductively Coupled Plasma-Mass Spectrometer, ICP/MS)  
 7.1.1. 가 (ICP-MS(ELAN DRC, PerkinElemer, USA) DRC mode(Dynamic reaction gas cell))  
 (Hadde VCM-41, Sweden) 200 g  
 1

**ICP-MS를 이용한 총비소 분석**

1.0 g microwave heating block (ED 16, LabTech, USA) 85°C 30  
 1 mL 가 microwave digestion system 5 90°C 10 5  
 150°C 10 5

**Table 3. ICP-MS Condition for total arsenic analysis**

Parameter	Operating conditions
RF Power	1,300 W
Plasma gas flow	15.0 L/min
Nebulizer gas flow	0.91 L/min
Auxillary gas flow	1.45 L/min
Lens voltage	6.50 V
Ion monitored	As m/z 75, DRC mode

190°C 30 (Table 2). heating block 12  
 0°C 4 1-2 mL가 가 20 가 3  
 0.45 μm (Fig. 1). (Merck, Germany)  
 1,000 mg/L 5% 가 1.0 mg/kg working solution 0.1, 1.0, 5.0, 10.0, 25.0, 50.0 μg/kg

(Wang *et al*, 1991).  
 가 (Inductively Coupled Plasma-Mass Spectrometer, ICP/MS)  
 ICP-MS(ELAN DRC, PerkinElemer, USA) DRC mode(Dynamic reaction gas cell)  
 Table 3

**HPLC-ICP-MS를 이용한 비소 화학종 분석**

HPLC ICP-MS  
 HPLC-ICP-MS  
 , 5 mM , 1% (Choi *et al*, 2016; Chen *et al*, 2016; Rintala *et al*, 2014). Ronkart (2007)

**Table 4. HPLC-ICP-MS condition for arsenic species analysis**

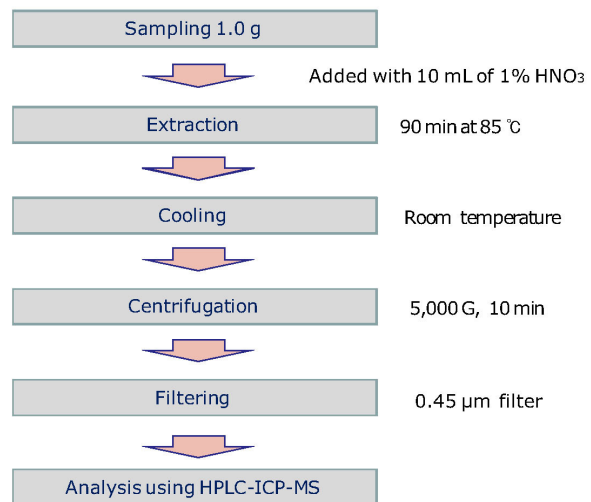
Instrument parameters	
HPLC	
As speciation	AsB <sup>a)</sup> , As(III), DMA, MMA, As(V)
Column	Anion exchange, Hamilton PRP X-100(4.1×250 mm, 10 μm)
Mobile phase A	12 mM ammonium carbonate
Mobile phase B	20 mM ammonium phosphate
Gradient profile	Run 1: 1 min / A 100%, Run 2: 7 min / B 100%
Flow rate	2.0 mL/min
Injection volume	50 μL
ICP/MS	
RF Power	1,300 W
Nebulizer gas flow	0.95 L/min
Auxillary gas flow	1.14 L/min
Plasma gas flow	16.0 L/min
Lens voltage	7.2 V
DRC mode	AsO 91, gas flow 0.5 mL/min

<sup>a)</sup>AsB: Arsenobetaine, As(III): Arsenite, DMA: Dimethylarsonic acid, MMA: Monomethylarsonic acid, As(V): Arsenate

pH 8  
 anion exchange column, 12 mM ammonium carbonate, 20 mM ammonium phosphate gradient  
 Table 4  
 1.0 g centrifuge tube, 1% 10 mL 가 5, 85°C 90  
 (TOMY MV-307, Hanil, Korea) 10 5,000 G, 0.45 μm  
 (Fig. 2). 5 1,000 mg/L 1% 10.0 mg/kg stock solution 가 1.0, 5.0, 10.0, 20.0 μg/kg

**시험법 검증**

(Limit of Detection, LOD) (Limit of Quantitation, LOQ)  
 , LOQ 2  
 LOQ 7  
 LOD=3.14 σ/S  
 LOQ=10 σ/S  
 (σ : , S : )  
 5

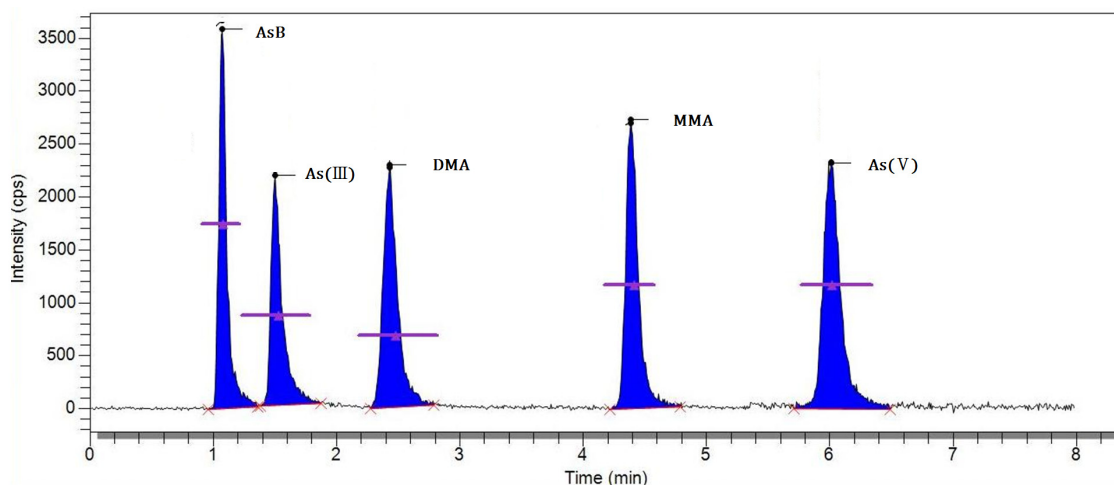


**Fig. 2. Flow chart for arsenic species analysis.**

(NIST) (Certified Reference Materials) 3  
 (Coefficient of Variation, %) C.V.  
 위해도 평가  
 12 188 가

**Table 5. Extraction efficiency by extract solution**

Extract	Species	AsB (%)	As (III)(%)	DMA (%)	MMA (%)	As (V)(%)
	Distilled water		39.6±3.7	43.8±6.5	68.2±7.1	63.8±8.8
5 mM malonic acid		77.1±8.0	76.6±8.2	82.5±4.9	79.6±6.8	93.4±10.1
1% nitric acid		97.8±8.2	106.4±6.9	95.6±10.1	97.1±8.4	102.9±6.5



**Fig. 3. HPLC-ICP-MS chromatogram for a standard mixture of 5 arsenic species. [AsB: Arsenobetaine, As(III): Arsenite, DMA: Dimethylarsinic acid, MMA: Monomethylarsinic acid, As(V): Arsenate]**

(Korea Centers for Disease Control & Prevention, 2012) 가 .  
 SPSS(ver 14.0, SPSS Inc., USA)  
 3  
 55 kg  
 JECFA  
 (Provisional Tolerable Weekly Intake, PTWI)  
 가 . PTWI 2010  
 15 µg/kg b.w./week가  
 PTWI 가  
 (determination approach) 가

(%) =

$$\frac{\text{JECFA (mg/kg b.w./week)}}{\text{(mg/kg b.w./week)}} \times 100$$

**결과 및 고찰**

비소 화학종 분리 시험법 확립

2 3  
 . Huang (2010)

90% MS ArCl ICP-tetramethyl  
 ammonium hydroxide phosphoric acid  
 swelling 가 0.28 M  
 가 .  
 , 5 mM  
 , 1%  
 95%  
 (Table 5).  
 pH  
 HPLC

Hamilton PRP-X100  
 (anion exchange column)  
 Raber (2012) HPLC  
 sodium sodium

acid ammonium , malonic  
 . 5 mM isocratic  
 arsenobetaine (AsB)  
 peak arsenite (As(III)) peak 가  
 (AsB) 가  
 ,  
 12 mM ammonium carbonate, 20 mM ammonium

**Table 6. Limit of detection (LOD) and limit of quantitation (LOQ)**

Item	Species	Total As ( $\mu\text{g}/\text{kg}$ )	AsB ( $\mu\text{g}/\text{kg}$ )	As (III) ( $\mu\text{g}/\text{kg}$ )	DMA ( $\mu\text{g}/\text{kg}$ )	MMA ( $\mu\text{g}/\text{kg}$ )	As (V) ( $\mu\text{g}/\text{kg}$ )
	LOD	0.13	0.87	0.73	0.76	0.98	1.24
	LOQ	0.43	2.87	2.41	2.51	3.23	4.09
	R <sup>2</sup>	1.0000	0.9984	0.9994	0.9991	0.9999	0.9986

**Table 7. Recovery and Coefficient of variation**

Item	Species	Total As	AsB	As (III)	DMA	MMA	As (V)
	Recovery (%)	98.6±0.7	94.5±1.3	102.1±1.8	98.4±2.3	94.3±2.0	96.2±2.4
	C.V. (%)	1.35	2.11	1.86	1.47	0.98	3.44

**Table 8. Total arsenic and inorganic arsenic concentration of each agricultural products**

Sample	N <sup>a)</sup>	Concentration (mg/kg)	
		Total Arsenic	Inorganic Arsenic
black rice	14	0.241±0.137 <sup>b)</sup> (0.029-0.449) <sup>c)</sup>	0.136±0.075 (0.017-0.278)
oat	14	0.031±0.019 (0.009-0.078)	0.017±0.011 (0.004-0.044)
millet	14	0.022±0.011 (0.008-0.044)	0.009±0.009 (N.D.-0.024)
mung bean	13	0.037±0.023 (0.011-0.084)	0.018±0.014 (N.D.-0.041)
barley	16	0.037±0.014 (0.007-0.063)	0.021±0.011 (N.D.-0.042)
sorghum	18	0.038±0.025 (0.007-0.104)	0.021±0.017 (N.D.-0.058)
adlay	14	0.033±0.013 (0.008-0.064)	0.019±0.009 (N.D.-0.041)
foxtail millet	16	0.045±0.019 (0.017-0.080)	0.026±0.014 (N.D.-0.051)
glutinous rice	12	0.267±0.090 (0.126-0.442)	0.149±0.056 (0.054-0.254)
soy bean	16	0.044±0.025 (0.014-0.109)	0.024±0.015 (N.D.-0.053)
adzuki bean	16	0.028±0.011 (0.014-0.053)	0.014±0.008 (N.D.-0.028)
mixed grain	25	0.183±0.045 (0.086-0.262)	0.110±0.035 (0.041-0.162)
Total	188	0.085±0.098 (0.007-0.449)	0.048±0.057 (N.D.-0.278)

N.D. : not detected

<sup>a)</sup>N, Number of sample

<sup>b)</sup>Mean value±SD (standard of deviation)

<sup>c)</sup>Concentration range (minimum-maximum)

phosphate gradient (Fig. 3).

**시험법 검증**

(Limit of Detection, LOD) (Limit of Quantitation, LOQ) LOQ  
LOQ 2 7

0.1, 1.0, 5.0, 10.0, 25.0, 50.0  $\mu\text{g}/\text{kg}$   
1.0, 5.0, 10.0, 20.0  $\mu\text{g}/\text{kg}$   
, R<sup>2</sup> 0.998

(Table 6).  
가 0.43  $\mu\text{g}/\text{kg}$ , 2.41-4.09  $\mu\text{g}/\text{kg}$   
g/kg 가  
arsenite(As(III))  
arsenate(As(V))

(NIST 1568b, USA ; NMIIJ 7503-a, Japan)  
50.0  $\mu\text{g}/\text{L}$  가 3

94.3-102.1%, C.V.% (Coefficient of Variation) 5%  
AOAC (AOAC International, 2002) U.S. FDA  
(Patrick *et al.*, 2015) (Table 7).

**잡곡 중의 총비소 농도 분석**

ICP-MS  
12 188  
0.085±0.098 mg/kg  
0.267±0.090 mg/kg, 0.241±0.137 mg/kg,  
0.183±0.045 mg/kg  
0.022±0.011 mg/kg 가  
(Table 8, Fig. 4). Choi (2010)

0.01 mg/kg 8 8



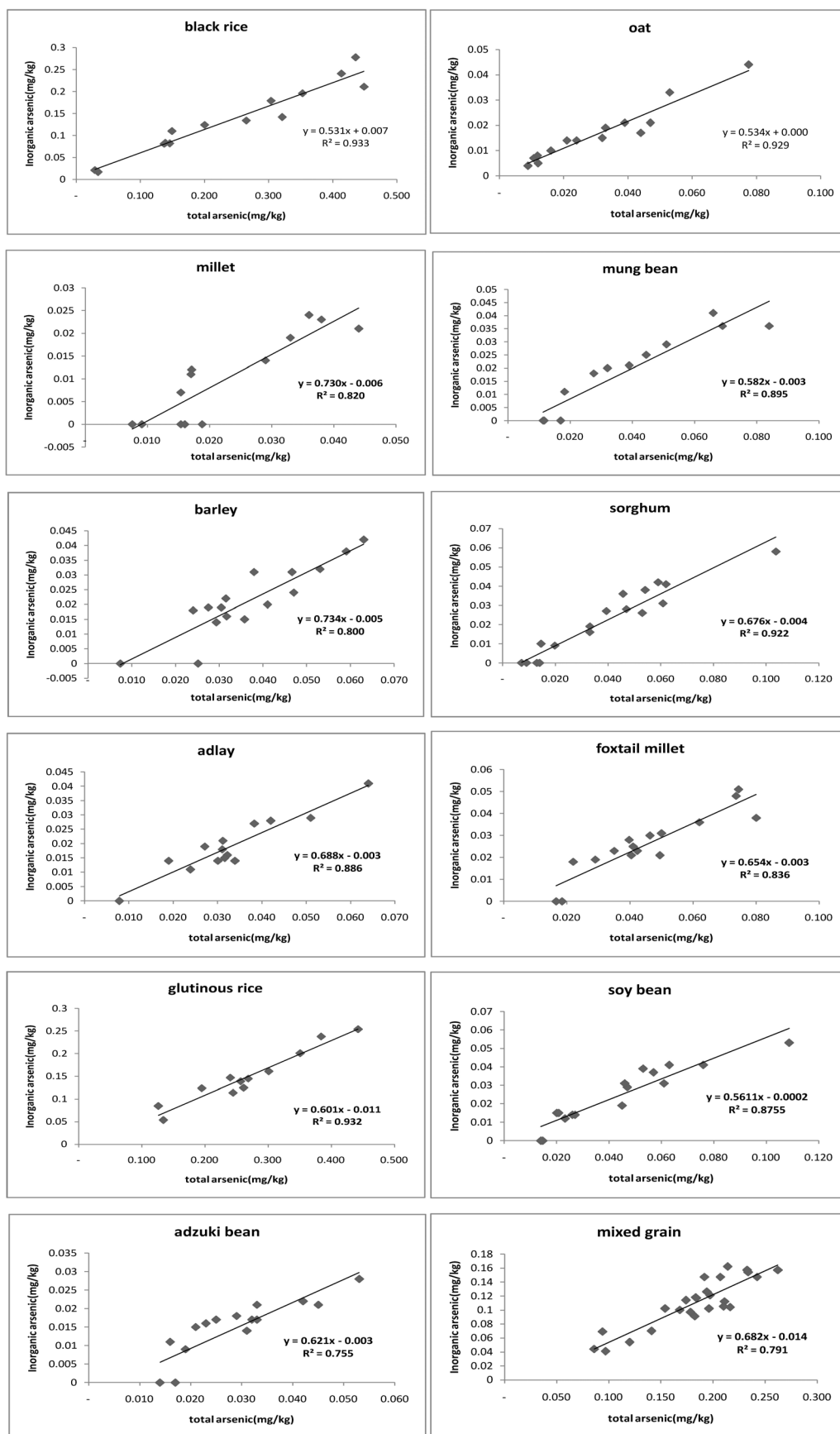


Fig. 5. Histogram of total arsenic and inorganic arsenic.



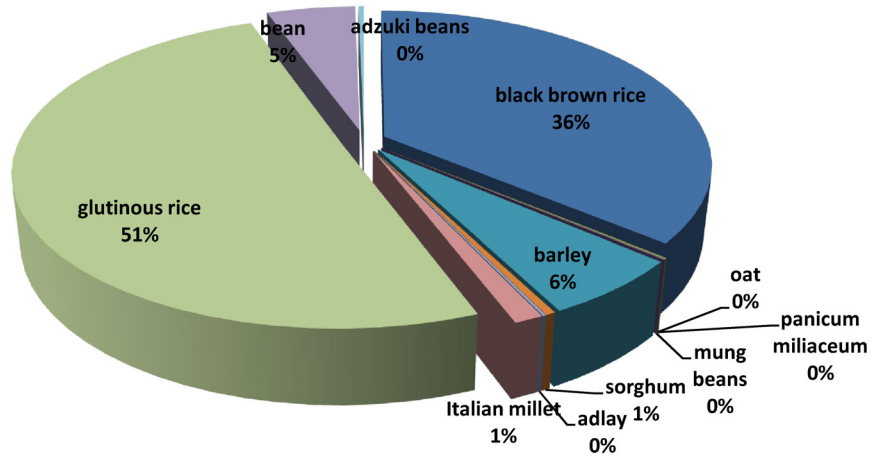


Fig. 6. Contribution rate of inorganic arsenic by agricultural products.

19.91  $\mu\text{g/day}$  가 , 1.07  $\mu\text{g/day}$ , 0.77  $\mu\text{g/day}$ , 0.13  $\mu\text{g/day}$ , 0.11  $\mu\text{g/day}$  가 PTWI 16.89% 가 , 0.267 $\pm$ 0.090 mg/kg, 0.241 $\pm$ 0.137 mg/kg, 0.183 $\pm$ 0.045 mg/kg, 0.149 $\pm$ 0.056 mg/kg, 0.136 $\pm$ 0.075 mg/kg, 0.110 $\pm$ 0.035 mg/kg 가 , 0.91%, 0.65%, 0.11%, 0.09% 가 (Table 9). 가 , 가 가 , (2012) 51%, 36%, 6%, 5% 가 , (Fig. 6). , 12 가 PTWI 18.69% 가 , 19.91  $\mu\text{g/day}$  가 , 1.07  $\mu\text{g/day}$ , 0.77  $\mu\text{g/day}$ , 0.13  $\mu\text{g/day}$ , 0.11  $\mu\text{g/day}$  가 PTWI 16.89% 가 , 0.91%, 0.65%, 0.11%, 0.09% 가 , (Table 9).

**요 약**

12 188 , HPLC-ICP-MS . HPLC ICP-MS . 1% , 1% , pH ammonium carbonate, ammonium phosphate gradient . AOAC .

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