

A Study on the Neosozine Residues in Rice Grain by Neutron Activation Method

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放射化分析法에 의한 米穀중 네오아소진 殘留분에 관한 研究

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Abstract

Residues of neosozine in rice grain were determined by neutron activation and colorimetric techniques. Twice application of the chemical before flowering did not lead to any increased residue level while 4-times application resulted in significant increase in the residue level up to 0.54-0.75 mg As_2O_3/kg . The partition ratio of arsenic residues into polished rice grain and bran was 73 : 27 in 100% polishing while most of the residues in the bran was transferred to oil cake fraction during solvent extraction, reaching up to 2.9 mg As_2O_3/kg . The neutron activation technique was advantageous because of its high sensitivity and the smaller sample amounts required for analysis.

Introduction

An organoarsenic fungicide, neosozine (ferric ammonium salt of methyl arsenate) is currently used for the control of sheath blight disease in paddy rice in Korea. Its usage has been steadily increasing in recent years, reaching 90 tons of active ingredient in 1978⁽¹⁾. However, this pesticide called a worrisome concern with its residue problem in the staple rice grain, due to the high toxicity of arsenic and its wide occurrence in soil. Substitute chemicals for the control of sheath blight disease in paddy rice are antibiotic fungicides such as polyoxin and validamycin which are known to be too expensive for the moment⁽²⁾.

Reports on the residue problem of organoarsenic

pesticides in crops are quite limited⁽³⁻⁵⁾. The residual property of neosozine in paddy rice and soil was studied by Fujimoto *et al.*⁽⁵⁾ and it provided the basis for the safe-use standard under agricultural environment of Japan. Bai⁽²⁾ also attempted to perform the same experiment in Korea although the scope and results were not comprehensive. It was felt that a more sensitive analytical method for the determination of arsenic was necessary in studying the residue problem.

This work was, therefore, undertaken to elucidate whether the conventional usage of neosozine in rice cultivation would cause any residue problem in terms of food safety from the time of application to the instant of consumption through polishing and oil-extraction processes. The results obtained under practical local agricultural conditions in

Korea by use of neutron activation analysis as well as conventional colorimetric method are presented here.

Materials and Methods

Field experiments

Paddy rice plants of an *Indica* type cultivar, "Milyang 24" were transplanted in an experimental paddy field divided into 9 plots of 300 m² each in Kyonggi-Do area as shown in Fig.1 and managed according to the conventional agricultural practices. A commercial 6.5% liquid formulation of neosozine was sprayed at the dose of 1.3 kg/ha each time according to the application schedule; twice (July 22 and August 11) or four times (July 21, August 4, 11 and 22). The flowering period of the crop lasted for a week beginning August 15. On October 4, rice plants of 13 stumps were cut from each plot and grains were collected, dehulled, and stored in polyethylene bags for residue analysis.

Polishing and oil-extraction procedures

Brown rice was polished with a laboratory mill to obtain 100% polished grain and rice bran. The bran was then extracted with *n*-hexane in a Soxhlet extractor for 24 hr to obtain oil and oil cake frac-

tions. The samples were subjected to the analysis of arsenic residues in triplicate by neutron activation technique.

Neutron activation analysis of arsenic

In order to increase the analytical sensitivity of arsenic present in biological samples, the neutron activation method described by Kimura *et al.*⁽⁵⁾ and Lee *et al.*⁽⁷⁾ was examined and modified to be suitable for the purpose of this study. The experimental details are described below.

One g of powdered rice sample packed in a polyethylene ampoule was irradiated in a pneumatic transfer system of the TRIGA Mark III Reactor (2MWt) installed at this Institute at a neutron flux of 1.5×10^{13} neutrons/cm²/sec for 20 min. After 24 hr, 5 ml of conc. HNO₃ and 3 ml of conc. H₂SO₄ were added to the irradiated sample. The mixture was warmed and conc. HNO₃ added until the mixture became clear. The digestion was then completed by adding 0.2 ml of perchloric acid and treated with 2 ml of saturated ammonium oxalate solution. After transferring the mixture to a gas generation bottle, 5 ml of conc. HCl, 2 ml of 15% KI solution and 0.4 ml of 35% SnCl₂ in conc. HCl were added. After 15 min, 3 g of sandy Zn was added to initiate gas generation. The gas was captured in a trap filled with 4 ml of silver diethyldithiocarbamate (Ag-DDC; 1 g in 200 ml pyridine) over the generation bottle during 1 hr.

Two ml aliquots of the trapping solution were counted in an Aloka model PC-IOE liquid scintillation counter at 72nd hr after irradiation.

A standard curve was constructed as follows and given in Fig.2. A 10 mg sample of As₂O₃ was irradiated, dissolved in 2.5 ml of 20% NaOH and diluted to 100 ml. The solution was then diluted to contain 0.025, 0.05, 0.1, 0.2, 0.5 and 1.0 μg As₂O₃/ml. To 1 ml each of the solution were added 5 μg of cold As₂O₃ as carrier and 1 g of rice powder and subjected to acid digestion, followed by gas generation and activity counting.

A linearity between the amount of arsenic and radioactivity was observed in the range of 0.025~0.5 μg of As₂O₃ by counting for 1 min and the sensitivity should be increased by extending the counting period. The lowest quantitation limit in

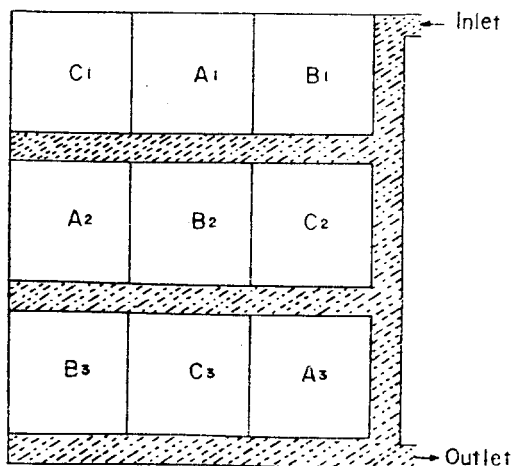


Fig. 1. Allocation of field plots for residue experiment

- A: Twice application on Jul. 22 and Aug. 11
- B: 4-times application on Jul. 21, Aug. 4, Aug. 11 and Aug. 22
- C: Control without application

this experiment was $0.025 \mu\text{g}$ of As_2O_3 , equivalent to 0.05 ppm in rice samples, and the reproducibility was satisfactory.

Colorimetric analysis of arsenic

The conventional colorimetric method of arsenic analysis was undertaken by the standard procedures of A.O.A.C.⁽⁶⁾, using 10 g of rice sample followed by digestion, gas generation and colorimetry with Ag-DDC. A standard curve is shown in Fig.3. A good linearity was observed in the range of 1~10

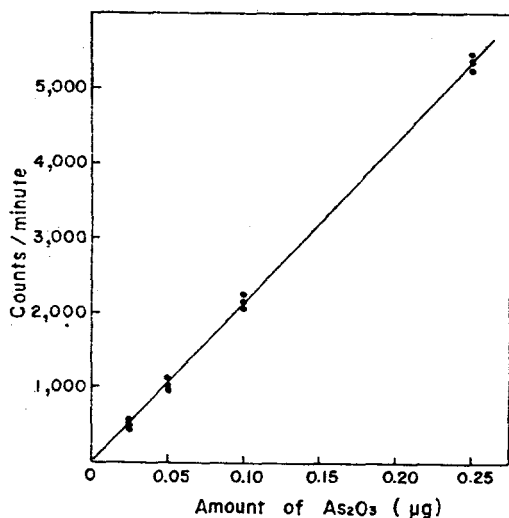


Fig. 2. Standard curve for the determination of arsenic by neutron activation method

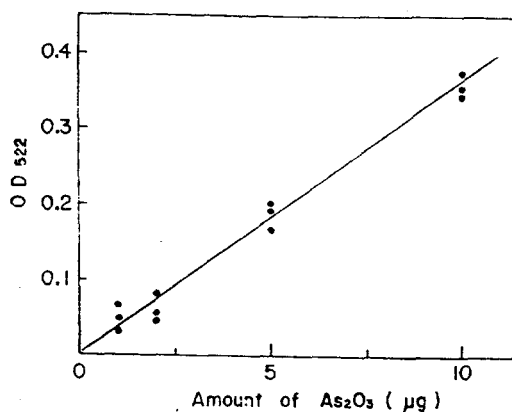


Fig. 3. Standard curve for the determination of arsenic by colorimetric method

μg of As_2O_3 , where the lowest quantitation limit was about $1 \mu\text{g}$, equivalent to 0.1 ppm in rice samples.

Results and Discussion

Arsenic residues in brown rice

Residues of total arsenic in brown rice samples after application of the organoarsenic fungicide, neoasozine, during the growth of paddy rice are shown in Table 1. It may be seen that twice application before flowering of the rice plants did not cause any significant increase in the residue level as compared with background level without application of around $0.15 \text{ mg As}_2\text{O}_3/\text{kg}$. However, 4-times application of the chemical until the time of flowering period resulted in a 4~5 fold increase of the residue level, reaching $0.54\sim0.75 \text{ mg As}_2\text{O}_3/\text{kg}$ air-dry matter.

Table 1. Arsenic residues in brown rice by field experiment*

(Unit: $\text{mg As}_2\text{O}_3/\text{kg}$)		
Treatment	Activation analysis	Colorimetric method
Control without application	0.137 ± 0.040	< 0.10
Twice application	0.157 ± 0.028	< 0.10
4-times application	0.543 ± 0.016	0.747 ± 0.037

* Data are means \pm standard deviation of nine determinations. Treatments were made in triplicate plots and residues were analyzed in triplicate for each plot.

Results obtained from both techniques were in good agreement. However, the neutron activation technique may have advantages over the colorimetric method because of its high sensitivity and the small sample quantities required for analysis. This was well demonstrated in this experiment since the background and low-level residues were near the lowest quantitation limit of the colorimetric method, which needs more sample (10 times). It is generally known that the detection limit for total arsenic is 0.1 ng in neutron activation analysis while it is $5\sim 250 \text{ ng}$ in colorimetric methods⁽⁹⁾.

Arsenic residues found after 4-times application of neoasozine may approach the lower tolerance limit of 1 ppm As_2O_3 (or 0.75 ppm As) since advanced countries set the residue limit of 1 or 3.5 ppm As_2O_3 depending on the commodity of fruits and

vegetables. Korean government established a tentative residue limit of 3.5 ppm As_2O_3 for brown rice. It is, therefore, suggested that the use pattern of the chemical be restricted to twice-application before flowering time of paddy rice.

Partitioning of arsenic residues in polishing and oil extraction processes

Brown rice samples obtained without application of neosozine as control and after 4-times application as highly contaminated sample were subjected

to polishing and oil extraction processes and analyzed the total arsenic by means of neutron activation technique. The results are given in Tables 2 and 3.

In polishing, the residue level of total arsenic was fortified 3~5 fold in rice bran while it was slightly decreased in polished rice grain. In particular, rice bran after 4-times application of neosozine reached the level of 2.3 mg As_2O_3/kg , which is higher than the lower tolerance limit of arsenic in foods (1 ppm). It is interesting to note that the higher level of contamination by neosozine may lead

Table 2. Partitioning of arsenic residues in the polishing process of brown rice

Sample	% Weight	Control without application		4-times application	
		Residue (mg As_2O_3/kg)	% Partitioning	Residue (mg As_2O_3/kg)	% Partitioning
Brown rice	100	0.165	100	0.723	100
Polished rice	91.6	0.084	53	0.565	73
Rice bran	8.4	0.803	47	2.338	27

Table 3. Partitioning of arsenic residues in the oil extraction process of rice bran

Sample	% Weight	Control without application		4-times application	
		Residue (mg As_2O_3/kg)	% Partitioning	Residue (mg As_2O_3/kg)	% Partitioning
Rice bran	100	0.803	100	2.338	100
Oil	21.4	0.117	3	0.133	1
Oil cake	78.6	1.014	97	2.909	99

to the accumulation of the residue in the endosperm portion of the grain rather than in the bran layer. This was well demonstrated in the % partitioning of the residue in the polished grain and rice bran; that is, the ratio in control rice was 53 : 47 while it was 73 : 27 in highly contaminated rice.

In oil extraction of the bran by solvent, the total arsenic residue goes mostly to the oil cake fraction, reaching 2.9 mg As_2O_3/kg , higher than the rice bran. It is very fortunate that the arsenic residue does not tend to be concentrated in the oil fraction. However, oil cake consumed as animal feed may contain very high arsenic residues even in the control sample without application and special attention should be paid on the toxicological problem. It is strongly suggested that the pesticide neosozine be restricted so as to be used under the recommended safe-use standard.

In conclusion, the data suggest that the products consumed by the human as grain and oil contained arsenic residues below the tolerance limit and are unlikely to present any toxicological hazard to the consumer. On the other hand, relatively high arsenic residues were found in the oil cake serving as animal feed and should be carefully evaluated in the light of the toxicological data.

요 약

有機砒素系 殺菌劑인 네오아소진의 米穀중 殘留量을 방사화 분석법 및 비색법으로 측정하였다. 수도재배중 開花前 2회 살포는 玄米중 잔류수준을 증가시키지 않았으나 4회 살포는 0.54~0.75 mg As_2O_3/kg 의 잔류수준까지 4~5배로 크게 증가시켰다. 현미 搗精중 白米 및 쌀겨로의 잔류분 分配比는 73 : 27이었고 쌀겨중 잔류분의 대부분은 용매에 의한 추출과정중 油粕에 이동

하여 2.9 mg As₂O₃/kg 수준까지 도달하였다. 放射化分析法은 比色法에 비하여 感도가 높고 分析試料가 少量 요구된다는 長點을 가지고 있었다.

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