# Determination of Selenium in Se-enriched Rice by Slurry Sampling Electrothermal Atomic Absorption Spectrometry

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**Abstract**

In the present work, determination of total selenium in Se-enriched rice was performed by slurry sampling electrothermal atomic absorption spectrometry (ETAAS) using palladium (II) nitrate as a chemical modifier. Optimized pyrolysis and atomization temperatures were 800 and 2,500 °C, respectively. Under the optimized conditions, the limit of detection (LOD) and quantitation (LOQ) were 4.72 μg $L^{-1}$ and 15.72 μg $L^{-1}$, respectively. Characteristic mass ($M_0$) of Se by ETAAS was 33.71 pg. Percentage - recoveries were in the range of 99 to 108 %. Concentration of selenium in regular rice was 0.4±0.2 μg $g^{-1}$. The selenium contents of rice were increased to 1.12-3.16 μg $g^{-1}$ by foliar application of Se-enriched fertilizer in the forms of sodium selenite and sodium selenate. In addition, the proposed technique was applied to the determination of selenium concentration in selenium supplements with satisfactory results.

**Keywords**: Selenium, Se-enriched rice, Slurry sampling, Electrothermal atomic absorption spectrometry (ETAAS)

## INTRODUCTION

Selenium (Se) is a naturally occurring and essential trace element in several metabolic pathways. The role of selenium in the prevention was established as a number of degenerative conditions including cancer, inflammatory diseases, cardiovascular disease, neurological diseases, aging, and infections (Ip, 1998; Reader's digest, 2000). Most of these effects are related to the function of selenium in the antioxidant enzyme systems. Selenium can be found in vegetables, grains, meats, seafood and nuts. In recent years, selenium-enriched yeast was produced in the form of selenium supplement that could increase the activity of the selenoenzymes. The studied benefits were reported in cancer prevention on the immune response and on HIV infection (Rayman, 2004). However, daily selenium intake for human life should be not more than 400 μg/day (Institute of Medicine, 2000). Rice, ordinary Thai cuisine, was reported in its selenium content approximately 5.0±1.1 μg/100 g (Sirichakwal et al., 2005). Determination of low concentration of selenium in rice using a sensitive technique is necessary. Nonetheless, suitable sample preparation technique must used to prevent loss and contamination during sample pretreatment procedure.

The determination of selenium in rice and other biological samples has been normally performed by using various sensitive analytical methods, such as inductively coupled plasma atomic emission spectrometry, electrothermal atomic absorption spectrometry (ETAAS), hydride generation atomic fluorescence spectrometry (HGAFS), and inductively coupled plasma mass spectrometry (ICP-MS) (Chen et al., 2002; Silva et al., 2007; Taylor et al., 2004). These analytical techniques are usually achieved under high pressure acid digestion for sample preparation e.g. microwave digestion. The sample digestion procedure is generally complicated and time-consuming. Furthermore, concentrated acid can be contaminated and high pressure can cause loss of analyte.

Slurry sampling is an alternative sample preparation technique for ETAAS. It was operated through simple reagents, no heat needed, and small amounts of samples were used. The process would be completed in a few minutes. It can be implemented directly on the autosampler cups in the ETAAS instrument. Moreover, real samples were directly analyzed. In addition, ultrasound-assisted extractions can be utilized to improve efficiency and time of separation of metals and metalloids from a variety of solid matrices (Montes-Bayón et al., 2006; Lavilla et al., 2008). In addition, slurry sampling was only reported to determine metals in rice flour. Selenium

determination in Se-enriched rice was rarely presented (González et a., 1999; Santos et al, 2002).

In this work, the sample preparation in slurry forms for the analysis by ETAAS was optimized including the temperature program for the determination by ETAAS technique. Slurry sampling technique was applied for total selenium determination in regular rice and Se-enriched rice samples. The accuracy of method was validated by determining selenium in some commercially available Se-enriched yeast supplements with the labeled concentration of 200 μg Se/tablet.

## MATERIALS AND METHODS

### Instrumentation

Measurements were performed on a Varian Model AA220Z electrothermal atomic absorption spectrometer equipped with an AS-50 autosampler. The selenium hallowed cathode lamp was operated at 10 mA (at the wavelength of 196.0 nm and spectral bandpass of 0.2 nm). The background Zeeman correction was applied. Longitudinal pyrolytically coated graphite tubes were employed.The temperature and time program for ETAAS operation are show in Table 1.

**Table 1** The optimized temperature program for direct ETAAS determination of total selenium

| Step No. | Temperature program | Temperature (°C) | Time (sec) | Gas Flow (L/min) |
|---|---|---|---|---|
| 1 | Drying | 85 | 5.0 | 0.5 |
| 2 | Drying | 95 | 20.0 | 0.5 |
| 3 | Drying | 120 | 10.0 | 0.5 |
| 4 | Pyrolysis | 800 | 5.0 | 0.5 |
| 5 | Pyrolysis | 800 | 1.0 | 0.5 |
| 6 | Pyrolysis | 800 | 2.0 | 0.0 |
| 7 | Atomization | 2500 | 0.8 | 0.0* |
| 8 | Atomization | 2500 | 2.0 | 0.0* |
| 9 | Clean up | 2500 | 2.0 | 0.5 |

*Read step

### Reagents

All chemicals used were of analytical-reagent grade. A stock standard solution of Se(1000 mg/L, Spectrosol) was used. A 65% w/v nitric acid (Carlo erba reagent) was used following appropriate dilution as extracting agent. Triton X-100 (Fluka) was used to suspend slurry and $Pd(NO_3)_2$ (Merck) was used as matrix modifiers. Three commercial available products of high Se-enriched yeast supplements with 200 μg of Se/Tablet (CVS® Pharmacy, Nature Made® and Schiff®) were used to evaluated the accuracy of the method.

### Sample preparation by slurry sampling ETAAS

Chai-Nat 1 Rice samples were cultivated by others in our laboratory at the local farmer field in 2008, Phitsanulok province. The foliar spraying with selenite or selenate fertilizer was thoroughly applied on the rice leaves twice (58 and 76 days) in the heading stage of rice growth at the level of 0, 80, and 160 g of Se/ha.

Rice grains were homogenized with an electronic blender. Fine powdered sample was sieved through the 75 μm mesh-size. For the optimization study, three fractions with different particle sizes were obtained from the samples (>250 μm, 125 to 250 μm and <75μm). Sieved samples were dried at 50 °C for 15 h and stored in desiccator until use.

Slurry sampling was modified from the method as presented by Méndez et al (Méndez et al., 2002). A 20 mg sample mass was suspended in a 1.5 ml volume of solution (2% v/v $HNO_3$ + $10^{-4}$% v/v Triton X-100). The suspended sample was extracted in ultrasonic bath for 1 minute. 20 μL standard/sample and 5 μL of palladium nitrate as a chemical modifier were injected into the furnace after vortex mixed for 10 seconds. Calibration with Se aqueous standards (0, 10, 25, 50, 75, 100 μg $L^{-1}$) using integrated peak area absorbance as the analytical signal was performed.

For three type of Se-supplement yeasts, the same procedure was performed other than sample mass. 1.5 mg of homogenized yeast sample was suspended in a 10 ml volume of the same solution. In order to reduce high selenium concentration in yeast samples to the detectable range.

## RESULTS AND DISCUSSION

### Optimization

In order to establish optimum extraction conditions, the univariate optimization procedure was used. Variables were studied as follows: particle size (μm), sample mass (mg), $HNO_3$ concentration (% v/v), Triton X-100 concentration (% v/v), extraction time (min), vortex mixing time (sec), stability of slurry sediment (min), pyrolysis temperature (°C), and atomization temperature (°C), respectively. Results of each experiment were the average value of three replicates. Rice sample fertilized with selenite 80 g of Se/ha was used as the target sample for optimization purposes. The optimized conditions for slurry preparation and pretreatment temperature for selenium determination by ETAAS are shown in Table 2 and Figure 1.

**Table 2** Optimized condition for the determination of selenium by using slurry sampling technique with ETAAS detection

| Variable | Optimized conditions |
|---|---|
| Particle size (μm) | <75 |
| Sample mass (mg) | 20 |
| $HNO_3$ concentration (% v/v) | 2 |
| Triton X-100 concentration (% v/v) | $1.0\times10^{-4}$ |
| Extraction time (min) | 1 |
| Vortex mixing time (s) | 10 |
| Stability of slurry sediment (min) | Analyzed immediately after vortex mixed |
| Pyrolysis Temperature (°C) | 800 |
| Atomization Temperature (°C) | 2,500 |

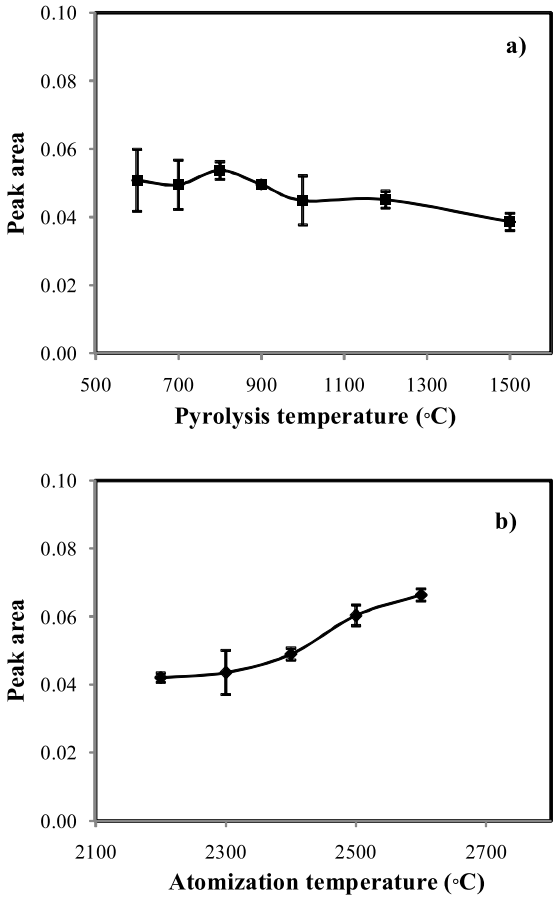


**Figure 1** Effect of a)pyrolysis temperature and b)atomization temperature on peak area signal of selenium by ETAAS

**Analytical characteristics**

Analytical characteristics were obtained under optimized conditions. Calibration curve was linear at least up to 80 μg $L^{-1}$. The equation of the linear calibration was y=0.0016x+0.0394 with $r^2$=0.9966. The limit of detection and the limit of quantitation of selenium were 4.72 μg $L^{-1}$ and 15.72 μg $L^{-1}$, respectively. The characteristic mass ($M_0$) for the determination of selenium was 33.71 pg defined as mass of element that give 0.0044 absorbance unit.

**Analysis of rice samples**

The optimized conditions were applied for selenium determination in Se-enriched rice. The results of selenium concentration calculated selenium content in unpolished rice grains are shown in Table 3. The selenium concentration of the regular rice was 0.39 ± 0.15 μg $g^{-1}$. The selenium contents of rice were increased in the range 1.12 to 3.16 μg $g^{-1}$ by foliar application of Se-enriched fertilizer in the forms of selenite and selenate compared with the control treatment with no selenium as shown in Figure 2. It was indicated that selenium content in whole rice grains could be increased three times by foliar fertilization with selenite and selenate. Especially, for selenate fertilizer could be increased selenium concentration to higher level. This finding is also agreed well as reported by Chen et al (Chen et al., 2002), who determined selenium in Se-enriched China rice by HGAFS. The selenium contents in this report were increased to 0.471-0.640 μg $g^{-1}$ when rice was applied with selenium fertilizers 20 g of Se/ha.

Moreover, the accuracy of the proposed method for determination of selenium was investigated in slurries of three commercial Se-enriched samples. Percentage - recoveries as compared to the concentration labeling of Se-enriched yeast supplements (200 μg/tablet), were in the range of 99 to 108 % as shown in Table 4. The results showed that the proposed technique could be accurately used for selenium determination in rice.

**Table 3** Selenium concentration in selenium-enriched rice (unpolished rice grains)

| Sample | Selenium concentration (µg/g, n=3) |
|---|---|
| Control | 0.39±0.15 |
| *Selenite* | |
| 80 g/ha | 1.30±0.19 |
| 160 g/ha | 2.82±0.16 |
| *Selenate* | |
| 80 g/ha | 1.12±0.08 |
| 160 g/ha | 3.16±0.40 |

**Table 4** Selenium content in selenium-enriched yeast supplements

| Se-enriched yeast supplements | Selenium content (µg/tablet, n=3) | % Recovery |
|---|---|---|
| Nature Made® | 197.3±5.2 | 98.6±2.6 |
| CVS® Pharmacy | 206.3±24.4 | 103.2±12.2 |
| Schiff® | 216.0±47.5 | 108.0±23.7 |

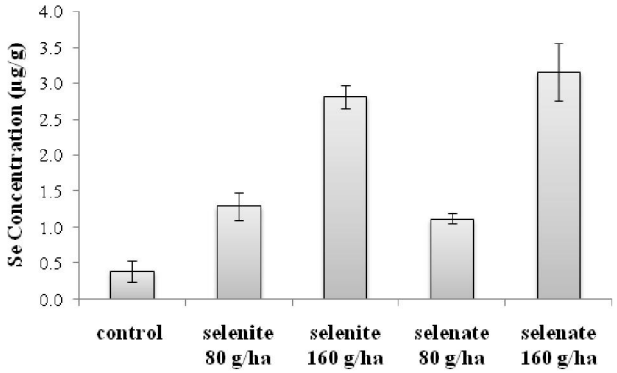


**Figure 2** Comparison of selenium concentration in selenium-enriched rice (unpolished rice grains) with different concentrations of foliar application of selenium fertilizers

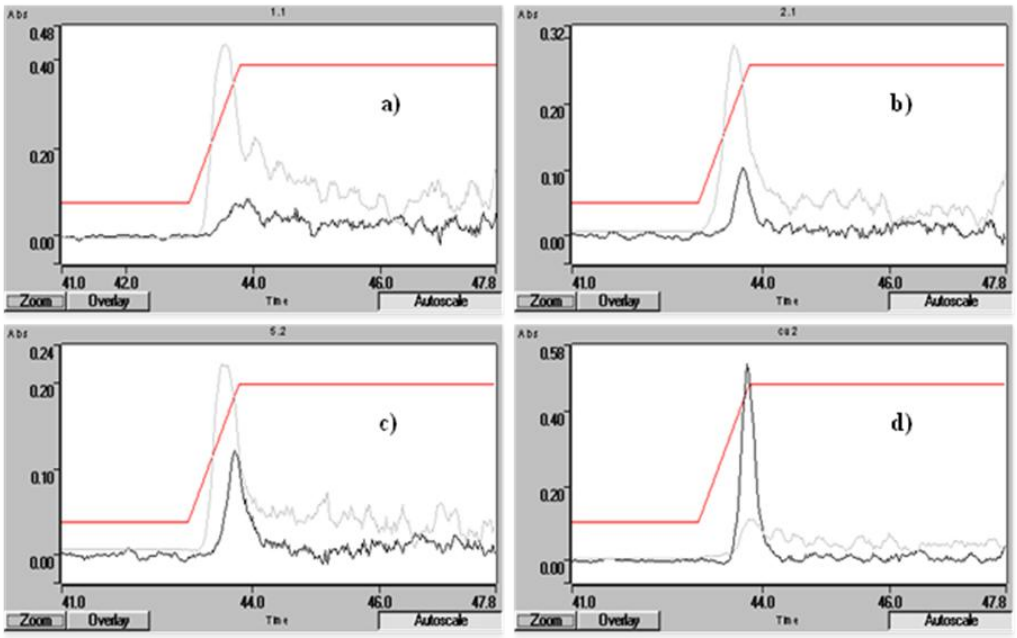


**Figure 3** ETAAS peak profiles of selenium in a) control rice, b) selenium-enriched rice (sodium selenite 160 g/ha), c) selenium-enriched rice (sodium selenate 160 g/ha), and d) Selenium-enriched yeast supplement (CVS® Pharmacy)

## CONCLUSIONS

Slurry sampling with ETAAS is an effective technique to determine low concentrations of selenium in rice and Se-enriched rice. The result indicated that selenium contents of rice were increased by foliar application of Se-enriched fertilizer in the forms of sodium selenite and sodium selenate (80, 160 g/ha). The satisfactory recoveries were obtained from the analysis of yeast selenium supplements. The proposed technique would be a suitable alternative method for selenium determination in rice samples, which could be reduce time-consuming and economy. In future work, antioxidant activity in rice and Se-enriched rice are going to research.

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