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)	1.0×10^{-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

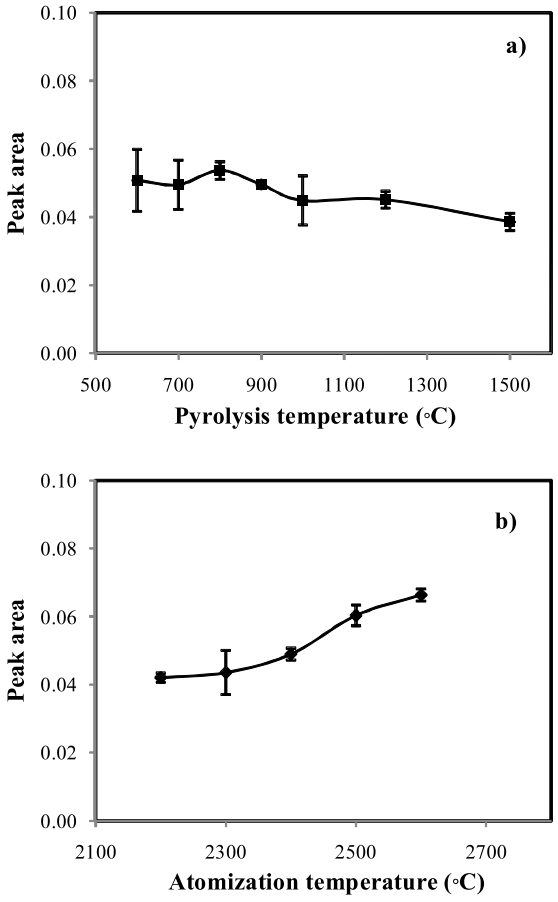

Figure 1 Effect of a)pyrolysis temperature and b)atomization temperature on peak area signal of selenium by ETAAS

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

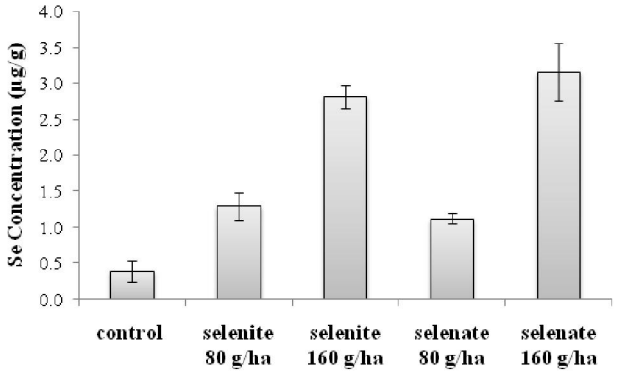

Figure 2 Comparison of selenium concentration in selenium-enriched rice (unpolished rice grains) with different concentrations of foliar application of selenium fertilizers

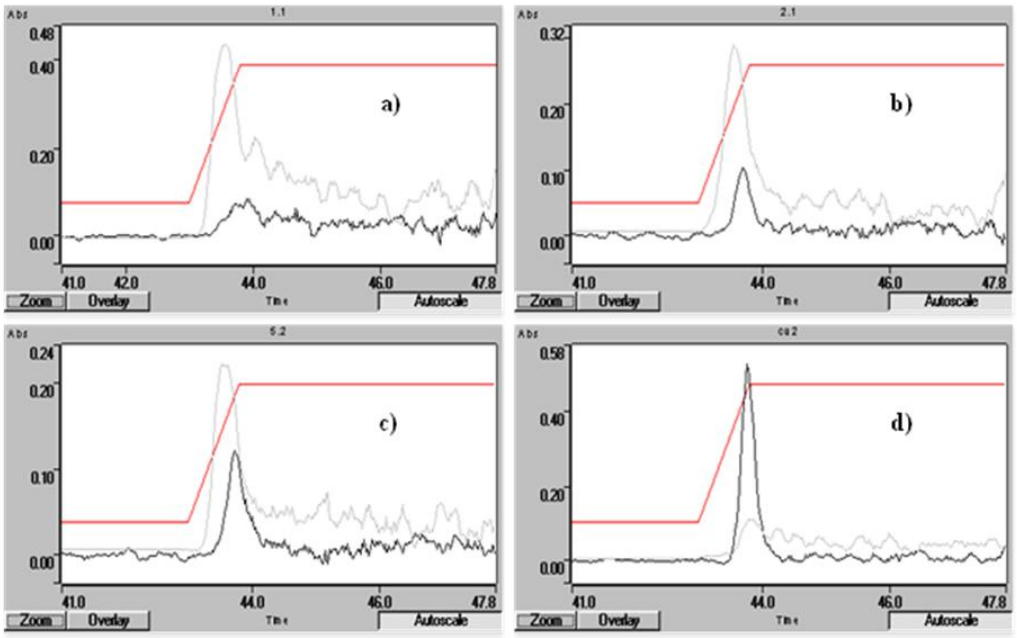

Figure 3 ETAAS peak profiles of selenium in a) control rice, b) selenium-enriched rice (sodium selenite 160 g/ha), c) selenium-enriched rice (sodium selenate 160 g/ha), and d) Selenium-enriched yeast supplement (CVS® Pharmacy)

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.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Chemistry, Faculty of Science, Naresuan University for providing the instrument facilities and scholarship from human resource development in science project (science achievement scholarship of Thailand, SAST) given to S.T. are acknowledged.

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