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Selenium Accumulation in Different Brown Rice Cultivars and Its Distribution in Fractions

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The goal of this paper was to study the accumulation of selenium (Se) in different cultivars of brown rice and its distribution in fractions. The results of the study showed that Se content in brown rice increased significantly ($P < 0.01$) as the external selenite or selenate concentrations increased from 10 to 180 μmol/L. In contrast, no significant influence ($P > 0.05$) on germination percentage and growth of sprouts was observed when the supplied Se was lower than 60 μmol/L. Moreover, selenite was easily transformed into selenoproteins to selenate. Based on this, ten brown rice cultivars were compared for Se accumulation. Likewise, significant difference ($P < 0.01$) was found among cultivars with respect to the capacity for Se accumulation. To understand the distribution of Se in selenized brown rice and its loss during milling, two cultivars with relatively higher ability to accumulate Se, namely, Zhendao 8 (Z8) and Xieyou 57 (X57), were selected for further study. The results showed that Se content was highest in the sprouts and decreased remarkably ($P < 0.01$) from the bran layers to the endosperm. In terms of Se loss during the milling procedure, 39.02% and 48.46% of Se were lost in Z8 and X57, respectively.

KEYWORDS: Selenium; brown rice; germination; selenium distribution

INTRODUCTION

Selenium (Se) is an essential element for human beings, and people acquire Se mainly from their diet. Rice is one of the most commonly consumed cereals in many countries, especially in Asia. However, Se content in rice is relatively low and fails to meet the daily dietary requirement. Consequently, some field experiments have been done in the hope of increasing Se content in rice grains by foliar spraying method (*1*−*4*). Though it is an effective way to increase Se content, nonetheless, it was associated with high costs. In addition, it was found that the long-term sprays may result in environment pollution (*5, 6*). Hence, it is necessary to find an alternative strategy for the production of selenized rice. During germination, rice seeds absorb Se. Thereafter, part of the Se could be assimilated into different seleno compounds by certain metabolic pathways. As a consequence, the Se levels in rice grains will be increased.

There is evidence that Se accumulation in plants is associated with Se chemical forms and plant cultivars. Research has been done with the objective of comparing the uptake among Se species in plants as well as among tissues (*5*−*8*). However, there are very few studies available showing how chemical species and dosage influence Se absorption, accumulation, and transformation in brown rice.

After selenized brown rice was developed, an interesting and important question still remains, namely, the location of Se allocation. Brown rice is composed of bran layers (6−7% of brown rice weight), embryo (2−3%), and endosperm (about 90%) (*9*). In order to obtain white rice, a mass of 10−15% was removed from brown rice. This resulted in considerable loss of nutrients such as lipids, proteins, vitamins, and minerals (*9*−*11*). Studies have been carried out on the contents of rice constituents in milling fractions obtained through multistep milling. Protein and mineral contents decreased from the outer bran layers to the endosperm (*10, 12*). Recently, Lamberts et al. (*11*) reported that bran contained much more yellow and red pigment than endosperm. Another study (*13*) provided detailed information on the distribution of zinc in different fractions by using different rice cultivars. However, no study has been done on the distribution of Se in selenized brown rice.

The objectives of this study were as follows: (i) confirm suitable chemical species and concentration range of Se for producing selenized brown rice, (ii) compare the abilities to accumulate Se among rice cultivars, and (iii) reveal the distribution of Se in different fractions of selenized brown rice. These objectives are in line with the study's desire to understand the loss of Se during milling as well as how to optimize the milling procedure.

MATERIALS AND METHODS

Grain Materials. Rough rice grains, japonica rice consisting of Yanjing 9 (Y9), Nanjing 42 (N42), Zhendao (Z8), Wuyun9522 (W9522), 86 You 8 (86Y8), and Wuyun 9520 (W9520), and hsien rice consisting of II You 838 (II838), K You 818 (K818), Xieyou 57 (X57), and Fengyou 22 (F22) were used in this study. These cultivars were

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Table 1. Instrumental Operating Conditions for HG-AFS

parameter	
high voltage of PMT (V)	−300
lamp current (mA)	80
atomizer temp (°C)	200
atomizer height (mm)	8
gas type	argon
carrier gas flow rate (mL min^{-1})	400
shield gas flow rate (mL min^{-1})	900
injection volume (mL)	2
dwell time (s)	1
read time (s)	10
read method	peak area
measurement method	stand curve

purchased from the Jiangsu Academy of Agricultural Science (JAAS). It must be noted that these cultivars are widely cultivated in the studied region. The brown rice was obtained after the removal of the husk using a hulling machine (JGMJ8098, Shanghai Jiading Grain and Oil Instrument Co. Ltd., China).

Preparation of Selenized Brown Rice. Ten grams of mature brown rice were sterilized with 1.0% (v/v) sodium hypochlorite for 30 min. Thereafter, it was washed three times with ultrapure water, and then it was well-distributed in a 15-cm Petri dish with two layers of filter paper. Fifteen milliliters of solution containing either selenite (10, 20, 30, 60, 90, and 180 μmol L^{-1}, respectively) or selenate (10, 20, 30, 60, 90, and 180 μmol L^{-1}, respectively) was added into the Petri dish. Subsequently, the brown rice was incubated in darkness at 32 °C for 48 h. During germination, an additional 3 mL of the corresponding Se solutions was added every 12 h. The control was incubated with ultrapure water under the same conditions.

Determination of Germination Percentage and Length of Sprouts. A grain was considered germinated when the radicle had pierced the seed coat. The findings show results due to the germination percentages obtained in three replicates ± SD (100 seeds each). Likewise, the length of sprouts was measured using a micrometer to an accuracy of 0.02 mm. Moreover, it had three replications of 20 seeds each.

Milling. Selenized brown rice was dried at 60 °C to a constant weight. One hundred grams of sample was successively milled in triplicate for 10, 20, 30, 40, and 50 s with a laboratory-scale milling machine (JGMJ8098, Shanghai Jiading Grain and Oil Instrument Co. Ltd., China). During milling, brown rice is subjected to abrasive or friction pressure to remove bran layers, resulting in different of degrees of milling (DOM). The DOM is defined as the percentage weight of brown rice removed in milling. After each 10 s milling, the milling machine was cleaned carefully and the abraded materials were collected to determine Se content in fractions. In another experiment, 100 g of samples was milled for 10, 20, 30, 40, and 50 s, respectively. The abraded materials and the white rice were collected to determine Se content and the DOM.

Se Determination. After germination, the selenized brown rice was washed with ultrapure water three times to remove the remaining Se on the surface and then dried at 60 °C to a constant weight. One gram of sample was digested with 5 mL of a mixture of HNO_3 and $HClO_4$ (v/v, 4:1) at 130 °C for 1 h. After cooling, 5 mL of concentrated HCl was added and incubated at 115 °C for 20 min. To avoid Se volatilization, reflux condensation equipment was employed. Subsequently, a clear solution was obtained. The solution was then transferred to a volumetric flask and allowed to rise to 100 mL with the addition of ultrapure water. The digested product was used for the determination of the total Se (T-Se) by hydride generation atomic fluorescence spectrometry (HG-AFS). The measuring conditions were according to Wu et al. (*14*) with some modifications (**Table 1**).

To determine the Se incorporated with proteins (P−Se), the semipermeable membrane device (SPMD) technique was used (*15, 16*). Approximately 0.3 g of the powdered selenized brown rice was transferred into a pretreated dialysis bag (15 cm long and 4.4 cm wide in plat) with a molecular weight cutoff of 3500 Da. Therefore, in this study, the P−Se denotes the selenoproteins whose molecular weights are higher than 3500 Da. After being sealed, the dialysis bag was placed in a beaker with 50 mL of ultrapure water and dialyzed at 37 °C for 7 days. During dialysis, the dialysate (ultrapure water) was replaced every 12 h. All of the dialysate was collected and concentrated for Se determination. In order to increase the rate of diffusion, the dialysis device was shaken at a speed of 150 rpm. After diffusion, the sample was dried with a freeze-drying system (Labconco, USA) for 24 h. The residual Se in the dialysis bag (defined as Se_1) and the Se in the dialysate (defined as Se_2) were determined respectively by the HG-AFS.

Meanwhile, approximately 0.3 g of the powdered selenized brown rice and 0.03 g of protease XIV (Sigma) were placed in a 15 mL centrifuge tube. The protease XIV is a nonspecific protease, which could break peptide bonds in proteins. After addition of 5 mL of ultrapure water, the sample was shaken at 37 °C for 24 h. After enzymatic hydrolysis, the sample was transferred into the dialysis bag and dialyzed for 7 days under the same conditions as described above. The residual Se in the dialysis bag (defined as Se_3) was determined by the HG-AFS.

The P−Se was calculated by the difference between Se_1 and Se_3, and the sum of Se_2 and Se_3 was given as the Se nonincorporated with proteins (NP-Se).

Statistical Analysis. The analysis of the variance was performed with the Statistical Analysis System software 8.2 (SAS, USA). Differences among means were evaluated using Duncan's multiple range test. Furthermore, Pearson's correlation coefficient values were determined at a significance level of $P < 0.05$.

Table 2. Effect of Selenium on Germination and Sprouts Growth of Brown Rice[a]

selenium conc (μmol/L)	germination (%)		length of sprouts (mm)	
	selenite	selenate	selenite	selenate
0	98.7 ± 0.3[a]	98.7 ± 0.3[a]	5.97 ± 0.50[a]	5.97 ± 0.50[a]
10	97.8 ± 0.7[a]	97.8 ± 0.6[a]	5.94 ± 0.81[a]	5.97 ± 0.94[a]
20	97.4 ± 1.1[a]	97.9 ± 0.6[a]	5.88 ± 0.80[a]	5.90 ± 0.71[a]
30	96.8 ± 1.7[a]	98.0 ± 0.4[a]	5.73 ± 0.75[a]	5.76 ± 0.68[a]
60	97.8 ± 0.3[a]	96.8 ± 1.1[a]	5.40 ± 0.77[a]	5.51 ± 0.69[a]
90	88.9 ± 2.0[b]	91.3 ± 3.3[b]	4.54 ± 0.80[b]	4.66 ± 0.93[b]
180	83.8 ± 3.9[c]	89.1 ± 2.3[b]	4.07 ± 0.37[b]	4.19 ± 0.34[b]

[a] The results are expressed as mean ± SD with three replications. Values within a column followed by the same superscript letter are not significantly different ($P < 0.05$). Brown rice germinated at 32 °C for 48 h, with different concentrations of external selenite or selenate.

RESULTS

Germination Percentage and Growth of Sprouts. Table 2 shows the germination percentage and the growth of sprouts after germination with a range of concentrations of selenite or selenate. Selenite and selenate had no significant influence ($P > 0.05$) on the germination percentage when the concentrations were lower than 60 μmol/L. However, the germination percentage was significantly decreased ($P < 0.01$) when the concentration of selenite or selenate was 90 or 180 μmol/L. Similar results were observed on the growth of sprouts. There was no significant effect on the growth of sprouts when the supplied Se concentration was lower than 60 μmol/L ($P > 0.05$). In contrast, an inhibitory impact was observed when the concentrations were higher than 60 μmol/L ($P < 0.01$). This suggested that, when producing selenized brown rice, Se concentration should be limited to lower than 60 μmol/L. Additionally, no significant differences were found in germination percentage ($P > 0.05$) and growth of sprouts ($P > 0.05$) between the chemical species of Se.

Accumulation of Se in Brown Rice. Figure 1 shows the levels of T-Se, NP-Se, and P-Se, and the ratio of P-Se to T-Se in brown rice germinated with different concentrations of selenite or selenate. It is clear from **Figure 1**A that T-Se content increased remarkably ($P < 0.01$) with an increase in the supplied

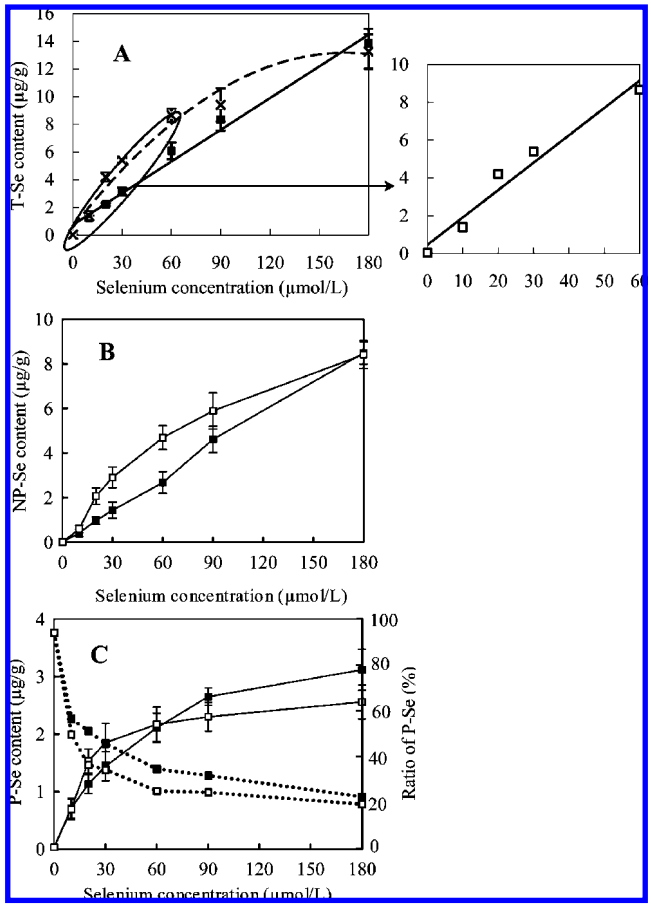

Figure 1. T-Se (**A**), NP-Se (**B**), and P-Se content (**C**, solid) and the ratio of P-Se to T-Se (C, dotted) in selenized brown rice. Selenized brown rice is obtained by germination at 32 °C for 48 h, with different concentrations of external selenite (■) or selenate (□). Values are the means of triplicate analyses. Error bars show the standard deviation. T-Se, total selenium; NP-Se, selenium nonincorporated with proteins; P-Se, selenium incorporated with proteins.

concentration of selenite or selenate. In the control, the T-Se content was 0.030 μg/g. However, when germinated with 180 μmol/L selenite and selenate, it reached 13.85 and 13.26 μg/g, respectively. T-Se content showed a Michaelis−Menten-type concentration-dependent kinetics curve for selenate-supplied grains ($R^2 = 0.974$, $P < 0.01$), although it showed a linear correlation for the selenite-supplied grains ($R^2 = 0.985$, $P < 0.01$) (**Figure 1A**). In the range of 0 to 60 μmol/L, the T-Se content in brown rice that was treated with selenite or selenate all increased linearly alongside an increase in the Se concentration ($P < 0.01$). No significant differences were found in the scopes of both lines for selenite and selenate ($P > 0.05$).

P-Se levels increased significantly ($P < 0.01$) with an increase of the supplied Se concentration (**Figure 1C**). In the control, the P-Se content was 0.028 μg/g. However, after germination with 180 μmol/L selenite and selenate, it reached 3.12 and 2.56 μg/g, respectively. This indicated that both selenite and selenate could be metabolized into selenoproteins during brown rice germination. P−Se accumulation showed similar concentration-dependent kinetics curves for both selenite and selenate. In the range of 0 to 180 μmol/L, P-Se accumulations in brown rice were quadratically correlated with the supplied concentration of selenite ($R^2 = 0.986$, $P < 0.01$) and selenate ($R^2 = 0.876$, $P < 0.05$).

Taking into account the relationship between T-Se and P-Se content, the ratio of P-Se to T-Se was calculated (**Figure 1C**). It was clear that the ratio of P-Se decreased remarkably ($P < 0.01$) with the increasing concentration of supplied selenite or selenate. The ratio of P-Se in samples germinated with selenate was lower ($P < 0.01$) than that with selenite. Considering that P-Se is safer to human beings than inorganic Se, selenite was chosen for further investigation.

Effect of Cultivars on Se Accumulation. The data in **Table 3** show the levels of Se in ten brown rice cultivars under natural conditions and after germination with 60 μmol/L selenite. Under natural conditions, Se content ranged from 0.017 μg/g in W9520 to 0.043 μg/g in 86Y8 and with an average of 0.038 μg/g. The results fall within the range of Se in previously reported rice (*1*, *2*, *4*). Statistical analyses showed that there were significant differences ($P < 0.01$) in Se levels among cultivars. After germination with 60 μmol/L selenite at 32 °C for 48 h, the T-Se content varied approximately 2-fold between cultivars ($P < 0.01$). X57, W9520, and Z8 contained the highest Se content. This was followed by F22, Y9, and 86Y8. On the other hand, W9522, N42, and II818 had the lowest levels. Significant differences were also observed in P-Se levels ($P < 0.01$) and the ratio of P-Se ($P < 0.01$) between cultivars. There was significant correlation between levels of T-Se and P-Se in selenized brown rice ($r = 0.959$, $P < 0.01$). However, with respect to Se content, no significant correlation was found between natural and selenized brown rice ($r = -0.522$, $P > 0.05$). Based on the T-Se content and the ratio of P-Se, Z8 (short-grain) and X57 (long-grain) were chosen for further study.

Relationship between Milling Time and DOM. Figure 2 shows the effect of milling time on the loss of mass in different cultivars. Loss of mass increased significantly ($P < 0.01$) for both studied cultivars when the milling duration was extended from 0 to 50 s. However, it did not show a linear relationship between the mass loss and the milling time. Loss rates become less while milling time was longer than 40 s. Regression analysis was performed to determine the relationship between milling duration (*x*, s) and percentage of mass loss (*y*, %) in different cultivars. The quadratic equations are as follows (eq 1, Z8; eq 2, X57):

$$y = -0.0039x^2 + 0.413x - 0.303, \quad R^2 = 0.994 \qquad (1)$$

$$y = -0.0054x^2 + 0.554x + 0.133, \quad R^2 = 0.998 \qquad (2)$$

The relationship between milling duration and the percentage of mass loss fitted a polynomial equation of $y = ax^2 + bx + c$, which was in agreement with the previously reported results (*13*, *17*). Similarly, the percentage of outer layers that were removed at milling stages differed significantly ($P < 0.01$) among the cultivars. Each milling stage caused higher loss of weight from X57 than from Z8. When milling for 50 s, the loss of weight from X57 and Z8 was 14.63% and 10.61%, respectively.

Se Distribution in Selenized Brown Rice and Its Loss during Milling. Se contents in fractions of selenized brown rice are shown in **Figure 3**. The highest Se content was observed in sprouts, 62.48 μg/g for Z8 and 63.48 μg/g for X57. The values decreased significantly from the bran layers to the endosperm, while white rice had the lowest Se content. Se levels in brown rice (nonmilled) of Z8 and X57 were 7.28 and 7.76 μg/g, respectively. However, when milled for 50 s, the content was 5.17 and 4.71 μg/g, respectively. The results indicated that Se is nonuniformly distributed in selenized brown rice. Similar results were found in other nutrients in brown rice (*10*, *11*, *13*).

During the milling procedure, the dates of Se recovery are shown in **Table 4** and the loss of Se as a function of milling time is shown in **Figure 4**. In both cultivars, the loss of Se

Table 3. Levels of Selenium in Ten Brown Rice Cultivars under Natural Conditions and after Selenium Enrichment[a]

cultivars		natural	after enrichment				
		T-Se (μg/g DW)	T-Se (μg/g DW)	P-Se (μg/g DW)	NP-Se (μg/g DW)	ratio of P-Se to T-Se (%)	recovery (%)
Japonica rice	Y9	0.026 ± 0.002^{cd}	5.45 ± 0.46^{bc}	1.78 ± 0.15^{bcd}	2.72 ± 0.13^{c}	32.60 ± 2.72^{c}	82.5
	N42	0.038 ± 0.005^{ab}	4.35 ± 0.83^{de}	1.75 ± 0.15^{cd}	2.12 ± 0.11^{de}	40.15 ± 3.35^{ab}	88.9
	Z8	0.027 ± 0.002^{cd}	7.28 ± 0.65^{a}	2.91 ± 0.38^{a}	3.46 ± 0.30^{ab}	40.02 ± 5.15^{ab}	87.5
	W9522	0.019 ± 0.002^{e}	4.31 ± 0.21^{de}	$1.54 \pm 0.16d^{e}$	2.35 ± 0.15^{d}	35.73 ± 3.60^{bc}	90.2
	86Y8	0.043 ± 0.002^{a}	5.44 ± 0.32^{bc}	2.11 ± 0.21^{bc}	2.81 ± 0.21^{c}	38.73 ± 3.79^{abc}	90.4
	W9520	0.017 ± 0.002^{e}	7.59 ± 0.25^{a}	3.31 ± 0.38^{a}	3.79 ± 0.30^{a}	43.57 ± 4.97^{a}	93.5
Hsien rice	II838	0.036 ± 0.003^{b}	3.82 ± 0.37^{e}	1.22 ± 0.17^{e}	1.94 ± 0.13^{e}	31.94 ± 4.47^{c}	82.7
	K818	0.030 ± 0.004^{c}	4.95 ± 0.32^{cd}	2.21 ± 0.19^{b}	2.14 ± 0.14^{de}	44.58 ± 3.85^{a}	87.8
	X57	0.022 ± 0.004^{de}	7.76 ± 0.53^{a}	3.26 ± 0.25^{a}	3.70 ± 0.22^{a}	42.05 ± 3.21^{ab}	89.8
	F22	0.022 ± 0.003^{de}	6.12 ± 0.20^{b}	2.17 ± 0.19^{bc}	3.30 ± 0.18^{b}	35.46 ± 3.10^{bc}	89.3

[a] The results are expressed as mean ± SD with three replications. Values within a column followed by the same superscript letter are not significantly different ($P < 0.05$). Selenized brown rice was obtained by germination with 60 μmol/L selenite at 32 °C for 48 h. T-Se, total selenium; NP-Se, selenium nonincorporated with proteins; P-Se, selenium incorporated with proteins.

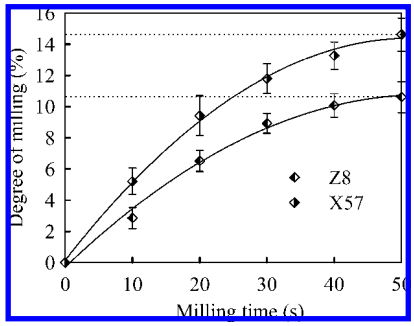

Figure 2. Effect of milling duration on degree of milling in different cultivars.

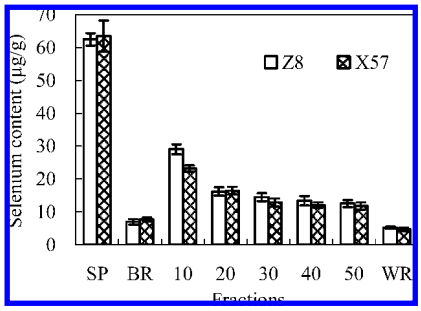

Figure 3. Selenium content in fractions of selenized brown rice. Values are the means of triplicate analyses. Error bars show the standard deviation. Selenized brown rice is obtained by germination with 60 μmol/L selenite at 32 °C for 48 h. SP, Sprouts of selenized brown rice; BR, selenized brown rice; WR, selenized white rice; 10, 20, 30, 40, 50, yield of abraded materials after every 10 s milling during successive milling from 10 to 50 s.

increased sharply ($P < 0.01$) with an increase in the milling time. This was especially true in the early milling stage (0−30 s). In addition, Se loss in X57 was higher compared to that in Z8 ($P < 0.01$). The regression equations for the relationship between milling time (x, s) and loss of Se (y, %) are as follows (eq 3, Z8; eq 4, X57):

$$y = -0.0209x^2 + 1.766x + 1.496, \quad R^2 = 0.983 \qquad (3)$$

$$y = -0.0258x^2 + 2.152x + 2.778, \quad R^2 = 0.968 \qquad (4)$$

DISCUSSION

The effect of Se on germination percentage and growth of sprouts was related to Se dosage. No visible detrimental impacts on germination and growth of sprouts were observed when the supplied Se was lower than 60 μmol/L. In contrast, toxic effects were found when the concentration was 90 or 180 μmol/L. This could be explained by the fact that Se could incorporate into the proteins of the amino acids in place of the equivalent sulfur amino acids, owing to the chemical similarity of Se and sulfur (*18*). The view was supported by the results reported by Tao et al. (*19*), where most of the Se was present in selenoproteins in selenized rice. In fact, our subsequent investigation revealed that a considerable amount of Se accumulated in brown rice was in the form of selenoproteins. This was especially true when the supplied Se was less than 60 μmol/L (**Figure 1** and **Table 3**).

Se accumulation in brown rice depended on the chemical species and concentrations supplied. Se accumulation was greatly correlated with the concentrations of Se supplied (**Figure 1A**). However, the curves for Se accumulation in selenite and selenate treated samples showed different patterns over concentrations. This indicated that selenite and selenate were absorbed and metabolized via independent mechanisms in rice grains. Similar results were reported in other plants (*7*, *20*, *21*). In addition, a higher ratio of P-Se to T-Se was observed in selenite-supplied compared to selenate-supplied brown rice (**Figure 1C**). This indicated that selenite was more easily transformed to P-Se compounds than selenate. The results are in agreement with other studies (*20*, *22*, *23*). It must be noted that the element selenium is both essential for many organisms and toxic at higher levels. Therefore, it is necessary to control the selenium-enrichment process strictly, especially the levels of Se supplied. Moreover, lower levels of external Se had higher rates of transformation from inorganic Se into organic Se.

Se content varied remarkably between cultivars. Under natural conditions, the variation may be attributed to differences in concentrations and chemical form of Se in the soil on which the rice plants were grown. Consequently, Se levels in rice can vary remarkably not only between countries but also between regions in a country. A study of 18 brown rice cultivars collected from 37 sites in Japan showed that the Se content ranged from 0.011 to 0.182 μg/g and that levels were related to soil Se content (*24*). Fang et al. (*4*) also observed great variations in levels of Se in rice obtained from 15 Chinese provinces, ranging from 0.003 to 0.049 μg/g. In addition, in the present study, significant differences were found between cultivars with respect to T-Se and P-Se accumulation when cultivated with 60 μmol/L selenite for 48 h. This observation will be helpful in developing better rice cultivars with relatively higher ability to accumulate Se. Similar results were reported by Zhang et al. (*5*, *6*) obtained in rice plants.

In the rice milling procedure, brown rice is subjected to abrasive or friction pressure to remove bran layers. About

Table 4. Recovery of Selenium during the Milling Procedure[a]

	Z8[b]			X57[b]		
milling time (s)	removed Se (μg)	residual Se (μg)	recovery (%)	removed Se (μg)	residual Se (μg)	recovery (%)
0	0	728.0	100	0	776.0	100
10	77.5 ± 11.7	590.3 ± 35.5	91.7 ± 6.5	132.7 ± 8.3	575.8 ± 36.1	91.3 ± 5.5
20	149.2 ± 9.5	507.6 ± 41.1	90.2 ± 6.9	185.1 ± 23.6	485.6 ± 31.5	86.4 ± 7.1
30	166.0 ± 15.2	480.8 ± 34.7	88.8 ± 6.8	277.1 ± 19.7	452.7 ± 42.7	94.0 ± 7.9
40	203.7 ± 12.0	462.3 ± 41.6	91.5 ± 7.3	310.8 ± 25.1	431.5 ± 34.4	95.7 ± 7.6
50	240.3 ± 16.7	444.0 ± 39.3	94.0 ± 7.6	316.3 ± 8.6	400.0 ± 38.9	92.3 ± 6.0

[a] The results are expressed as mean ± SD with three replications. [b] Brown rice was selenized by germination with 60 μmol/L selenite at 32 °C for 48 h, and 100 g of samples were used for the determination.

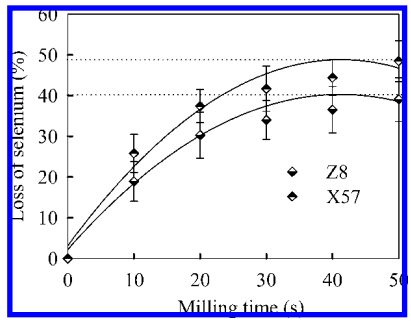

Figure 4. Effect of milling duration on loss of selenium in different cultivars.

10−15% of mass is removed from the outer layers (mainly bran) in order to obtain white rice. In the present study, the loss of weight was 10.61% for Z8 and 14.63% for X57 when the rice was milled for 50 s (**Figure 2**). Liang et al. (*13*) reported that when rice was milled for 50 s, about 5−6% of mass loss was achieved. The value was lower compared to the results reported by Singh et al. (*17*), where about 5−11% of bran was removed when milling for the same time. In another study, about 16% of mass loss was observed when milling for 50 s (*11*). These variations in milling degrees were due to the differences in morphological characteristics of rice grains such as aspect ratio, equivalent diameter, sphericity, and hardness, among others. This view was supported by the results reported by Prom-u-thai et al. (*25*), where DOM was significantly affected by morphological characteristics, in which grain length and length-to-width ratio made the largest contribution. In the present study, X57 is slender and the length-to-width ratio is higher than that for Z8. Consequently, more of the edges will be removed while subjected to the same milling time.

Owing to the nonuniform distribution of nutrients, though about 10−15% of mass was removed during milling, a considerable nutriment was lost. Lamberts et al. (*11*) reported that a major part of minerals was found in the bran, and proteins were mostly concentrated in the outer endosperm. When 15% of mass was removed (consisting of bran and outer endosperm), about 37.6% of total protein, 84.7% of minerals, and 9.2% of starch were lost. Prom-u-thai et al. (*25*) also observed that the content of iron was higher in the outer layer of brown rice compared to the endosperm. Some other literature also reported similar results (*10*, *13*, *17*). In this study, we found that Se levels decreased significantly from the outer layer to the endosperm (**Figure 3**), and 39.02−48.46% of Se was lost after being milled for 50 s. One reason for this could be that 12−15% of rice bran was composed of protein, higher than in endosperm (*10*, *26*, *27*), and a considerable amount of Se in selenized brown rice was incorporated with proteins (*19*).

From this study we conclude that external Se supply during germination is an effective way to increase Se level in brown rice. In addition, Se accumulation is associated with the chemical forms and dosages of supplied Se. These observations suggest that selenized brown rice can be obtained by selecting a suitable form and concentration range of Se. In the cultivars study, significant differences were observed between cultivars with respect to the capacity of Se uptake and accumulation. This information will be of help in producing selenized rice. Results of this study also showed that the levels of Se in selenized brown rice decreased significantly from the surface to the endosperm and a considerable amount of Se was located in the outer layer (10−15% of mass). Milling duration significantly influenced the Se content of white rice because some fractions of brown rice were removed which contained a significant amount of Se. This indicates that there is a potential to remove the outer layers of the brown rice while retaining its Se content as much as possible by optimizing the milling procedure.

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