JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



# Assessment of Selenium Bioavailability from High-Selenium Spirulina Subfractions in Selenium-Deficient Rats

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It was previously found that the bioavailability of Se from Se-rich spirulina (SeSp) was lower than that from selenite or selenomethionine when fed to Se-deficient rats. The present study examined the bioavailability of Se from SeSp subfractions: a pellet (P) issuing from the centrifugation of a suspension of broken SeSp and a retentate (R) resulting from ultrafiltration of the supernatant through a 30 kDa exclusion membrane. Animals were fed a torula yeast based diet with no Se (deficients) or supplemented with 75 $\mu$g of Se/kg of diet as sodium selenite (controls) for 42 days. Se-deficient rats were then repleted for 56 days with Se (75 $\mu$g/kg of diet) supplied as sodium selenite, SeSp, P, or R. During this period, controls continued to receive sodium selenite. Speciation of Se in subfractions showed that the majority was present in the form of high molecular weight compounds; free selenomethionine was only a minor constituent. Gross absorption of Se from sodium selenite, P, and R was not different and was higher than from SeSp. Only retentate allowed full replenishment of Se concentration in liver and kidney (as did sodium selenite) and glutathione peroxidase (GSHPx) activity in liver, kidney, plasma, and erythrocytes. The bioavailabilities of Se in retentate, as assessed by slope ratio analysis using selenite as a reference Se, were 89 and 112% in the tissue Se content and 106−133% in the GSHPx activities. SeSp and P exhibited a gross bioavailability of <100%. These results indicate that Se in retentate is highly bioavailable and represents an interesting source of Se for food supplementation.

**KEYWORDS: Selenium; bioavailability; glutathione peroxidase; high-selenium spirulina subfractions**

## INTRODUCTION

Selenium is an essential trace dietary nutrient for human beings and animals. It is an integral component of the enzyme glutathione peroxidase (GSHPx) (*1*) and other selenoenzymes or selenoproteins (*2*), which are involved in the removal of hydrogen peroxide and lipid peroxides produced during oxidative process in cells. This function helps to maintain membrane integrity (*3*) and the likelihood of propagation of further oxidative damage to lipids, lipoproteins, and DNA with the associated increased risk of conditions such as atherosclerosis and cancer (*3*). Selenium deficiency in humans may be related to other diseases such as ischemic heart disease (*4*), Keshan disease (*5*), multiple sclerosis (*6*), muscular dystrophy (*7*), rheumatoid arthritis (*8*), and cataracts (*9*). Consequently, Dietary Reference Intakes are very important and have established for Se an intake of 55 $\mu$g/day for both women and men (*10*). The amount of selenium in the diet and therefore the activity of selenoproteins depend to a great extent on the selenium concentration in the soil. Due to climatic and excessive artificial fertilization of soils, dietary selenium intake is generally low in Europe (*11*). Moreover, the metabolism of selenium varies according to the chemical form of selenium ingested; this determines its bioavailability (*12*), that is, intestinal absorption and subsequent tissue distribution and utilization. The major forms of selenium in foodstuffs are selenomethionine (SeMet) and selenocysteine (SeCys). Inorganic selenium salts are added to foods as supplements (*13*). In general, the bioavailability of selenium in SeMet, SeCys, and most plant materials appears to be reasonably good, whereas that of the Se in many animal products appears to be moderate and in some cases low (*14*). We have recently reported that selenium from selenium-rich spirulina was less effective and less bioavailable than selenium from sodium selenite and SeMet in repleting GSHPx activity and tissue selenium in most organs and tissues of Se-depleted

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10.1021/jf011646t CCC: $22.00 
Published on Web 05/29/2002

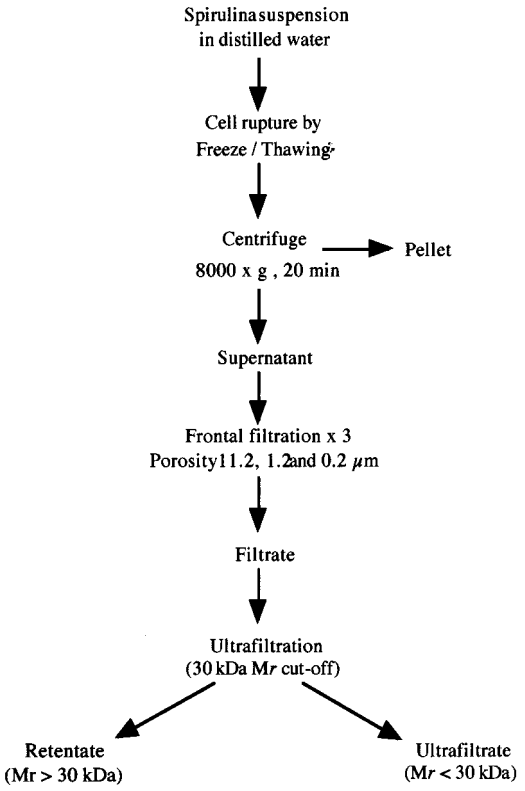


**Figure 1.** Schematic procedure for spirulina fractionation.

rats (*15*). Finley (*16*) reported that bioavailability can be used synonymously with biological usefulness; thus, due to its non-negligible effects, high-selenium spirulina possessed a biological usefulness and was an effective source of selenium that we have previously shown to have beneficial effects in rats fed high-cholesterol diets (*17*). Spirulina (*Spirulina platensis*) is a blue-green alga belonging to the cyanobacteria family, commercially available for human consumption and used as a functional food for humans (*18*). By way of aquatic medium, spirulina is an easily selenium-supplementable vegetable. Because bioavailability of selenium varies as a function of its chemical form, we decided in this study to separate different fractions from Se-rich spirulina and to examine the impact of these fractions on selenium-deficient rats.

## MATERIALS AND METHODS

**Selenium-Rich Spirulina Production.** The algae-fortification step was performed at Aquamer S.A. (Mèze, France). Algae were grown in a 130-L photobioreactor under continuous lighting on Zarouk's medium at 22 °C and pH 10.5 in the presence of selenium dioxide (under patent preparation). The medium contained 16.8 g/L $NaHCO_3$, 0.5 g/L $K_2HPO_4$, 2.5 g/L $NaNO_3$, 1.0 g/L $K_2SO_4$, 1.0 g/L NaCl, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.04 g/L $CaCl_2$, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 0.08 g/L EDTA, $2.86 \times 10^{-3}$ g/L $H_3BO_3$, $1.81 \times 10^{-3}$ g/L $MnCl_2 \cdot 4H_2O$, $0.22 \times 10^{-3}$ g/L $ZnSO_4 \cdot 7H_2O$, $7.9 \times 10^{-5}$ g/L $CuSO_4 \cdot 5H_2O$, $1.5 \times 10^{-5}$ g/L $MoO_3$, and $2.1 \times 10^{-5}$ g/L $Na_2MoO_4$ and was supplied with a light aeration (30 L/min) and with addition of 0.03% $CO_2$. At the end of the growth, the biomass was recovered and filtered through a 20-$\mu$m membrane, thoroughly washed with distilled water, frozen, and lyophilized.

**Spirulina Fractionation.** Fractions were isolated as shown in **Figure 1**. Selenium-rich spirulina (SeSp) were suspended in distilled water (50 g/L). Then, the suspension was submitted to two freezing/thawing cycles to induce cell breaking and centrifuged for 20 min at 8000*g*; the pellet (P) was recovered, washed two times with distilled water, frozen in liquid nitrogen, and lyophilized. The supernatant was submitted to three successive frontal filtrations (membrane porosities of 11.2, 1.2, and 0.2 $\mu$m, respectively). An ultrafiltrate (UF) was obtained by passing the filtrate, at 4 °C under nitrogen pressure, through an Amicon (Millipore S.A., Saint-Quentin-en-Yvelines, France) stirred ultrafiltration cell fitted with a 30000 $M_r$ exclusion membrane. The UF and retentate (R) were frozen in liquid nitrogen and lyophilized; the UF was not tested for Se bioavailability and served only to verify Se balance after fractionation.

**Characterization of Selenium Speciation in Fortified Spirulina.** Selenium speciation was characterized by a sequential extraction with reagent solutions meant to selectively leach different classes of selenium species into an aqueous phase. The extracts were subsequently characterized by size exclusion, anion-exchange, and cation-exchange HPLC with Se-specific detection by inductively coupled plasma mass spectrometry (ICP-MS) (*19*). A 0.2 g sample was taken for analysis. It was extracted sequentially with (a) 5 mL of hot water (85−90 °C) by agitation for 1 h for water soluble selenospecies, (b) 5 mL of 4% Driselase in 30 mM Tris-HCl buffer (pH 7.0) in the presence of 1 mM PMSF by agitation for 1 h at 25 °C for selenium associated with cell wall, (c) 5 mL of 30 mM Tris-HCl buffer (pH 7.0) containing 4% SDS by agitation for 1 h at 25 °C for water insoluble selenoproteins, and (d) 5 mL of phosphate buffer (pH 7.5) by incubation for 16 h at 37 °C with a mixture containing 10 mg of lipase and 20 mg of Pronase to free and break the residual selenoproteins. Selenium was determined (by ICP-MS) in the supernatant remaining after the centrifugation. The final residue was dissolved completely in 2 mL of 25% tetramethylammonium hydroxide (TMAH) in water to determine the residual Se. A 100 $\mu$L aliquot of extract (10-fold diluted) was injected on a Superdex Peptide HR 10/30 column (Pharmacia, Uppsala, Sweden) and eluted at 0.75 mL $min^{-1}$ with 30 mM Tris-HCl buffer (pH 7.5).

Anion-exchange chromatography was performed on a Hamilton PRPX-100 column at a flow rate of 1 mL $min^{-1}$ ; the solvents used for separation were as follows: solvent A, 5 mmol/L ammonium acetate adjusted to pH 4.7 with acetic acid; solvent B, 100 mmol/L ammonium acetate adjusted to pH 4.7 with acetic acid. Chromatographic conditions consisted of 5 min at 100% A, 35 min from 0 to 100% B and 20 min at 100% B. Cation-exchange chromatography used a Supelcosil SCX column at a flow rate of 1 mL $min^{-1}$ with 2 mmol/L phosphate at pH 6.0 (A) and 25 mM phosphate at pH 6.0 (B); elution was performed with a gradient consisting of 10 min at 100% A, 20 min from 0 to 100% B, and 20 min at 100% B.

$^{78}Se$, $^{80}Se$, and $^{82}Se$ were monitored on-line using an Elan 6000 ICP MS spectrometer (PE-SCIEX, Thornhill, ON, Canada). Total selenium was determined by standard addition at two levels corresponding to the selenium content in the sample. The HPLC-ICP-MS for Se speciaton analysis was performed using an HP1100 HPLC pump (Hewlett-Packard, Waldbronn, Germany) coupled to the Elan 6000 (Perkin-Elmer SCIEX, ON, Canada) via a Scott double-pass spray (4.6 × 250 mm × 10 $\mu$m) Hamilton PRPX-100 (Supelco, St. Quentin Fallavier, France), (4.6 × 250 mm × 5 $\mu$m) Supelcosil SCX (Supelco, St. Quentin Fallavier, France), and (10 × 300 mm × 13 $\mu$m) Superdex Peptide (Pharmacia) columns for anion-exchange, cation-exchange, and size exclusion chromatography, respectively.

**Animals and Diets.** Male Sprague−Dawley rats (Iffa Credo, L'Arbresle, France) weighing ~68 g were individually housed in suspended stainless steel metabolic cages in a temperature-controlled (22−24 °C) room with a 12 h light−dark cycle. Animals had free access to deionized water and were weighed weekly. They received a selenium-deficient torula yeast (TY) basal diet (**Table 1**) and were maintained by pair-feeding to the food intake of the group having the lowest intake over the experimental periods. On arrival and just before the beginning of the experimental period, 5 of the 90 rats were selected at random and sacrificed to evaluate tissue GSHPx activity and Se concentration to establish the baseline. At the start of the experimental period, the other 85 rats were divided into two dietary groups according to the average body weight: the control group (group C; $n = 20$) received the TY diet to which was added 75 $\mu$g of Se/kg as $Na_2SeO_3$. The entire experiment lasted 98 days with two distinguishable periods. The Se-depletion and Se-repletion periods consisted of 42 and 56 days, respectively. For the first 42 days (Se-depletion period) the remaining animals ($n = 65$) were fed the TY Se-deficient basal diet (Se-deficient dietary group; group SD), which contained 7 $\mu$g if Se/kg. At the end of this period, liver, kidney, and blood were removed from five animals

**Table 1.** Composition of Experimental Diets (Grams per Kilogram)[a]

| diet component | control diet (group C) | Se-deficient diet (group SD) |
|---|---|---|
| torula yeast[b] (N × 6.25) | 323 | 323 |
| DL-methionine | 1 | 1 |
| cornstarch | 344 | 344 |
| sucrose | 172 | 172 |
| cellulose | 50 | 50 |
| corn oil | 50 | 50 |
| mineral mix[c] | 40 | |
| mineral mix[d] | | 40 |
| vitamin mix[e] | 20 | 20 |
| total selenium (μg/kg) | 75.00 | 7.00 |

[a] All diets were formulated to contain 15% protein. [b] Torula yeast contained 7.42% N. [c] Mineral mixture contained (mg/kg of diet) the following: $CaHPO_4$, 17200; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; $Fe_2O_3$, 120; $FeSO_4 \cdot 7H_2O$, 200; trace elements, 400 ($Na_2SeO_3$, 0.165; $MnSO_4 \cdot H_2O$, 98; $CuSO_4 \cdot 5H_2O$, 20; $ZnSO_4 \cdot 7H_2O$, 80; $CoSO_4 \cdot 7H_2O$, 0.16; KI, 0.32; sufficient starch to bring to 40 g (per kg of diet). [d] Mineral mix as described above and free from $Na_2SeO_3$. [e] Vitamin mixture containing (mg/kg of diet) the following: retinol, 12; cholecalciferol, 0.125; thiamin, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamin, 0.1; *all-rac*-α-tocopherol, 340; menadione, 80; nicotinic acid, 200; choline, 2720; folic acid, 10; *p*-aminobenzoic acid, 100; biotin, 0.6; sufficient starch to bring to 20 g (per kg of diet).

from each group for Se bioassays and GSHPx activity in order to ascertain that the Se-deficient diet had induced a depleted Se status. The Se-repletion period lasted from day 43 to day 98: the animals from group SD were randomly divided into four dietary groups of 15 animals, which were fed a diet containing 75 μg of Se/kg either as sodium selenite, SeSp, P, or R. Five rats of each group were killed at days 7, 28, and 56 of the repletion period. Five animals from the control group were also killed at the same periods.

**Selenium Assay in Animal Tissues and Spirulina Fractions.** Samples were wet-ashed overnight with concentrated nitric acid in borosilicate test tubes placed in a hot block (Bioblock Scientific, Illkirch, Paris, France) at 110 °C. Selenium concentration was determined by ICP-MS using a Varian Vista spectrometer (Varian, Les Ulis, France).

**Analytical Procedures.** Animals were treated in accordance with the *Guide for the Care and Use of Laboratory Animals* (*20*). They were unfed overnight and anesthetized (pentobarbital 6%, 60 mg/kg of body wt) before tissues were excised. Kidneys were excised, washed in ice cold saline, blotted, weighed, and stored in liquid nitrogen. The liver was perfused with 1.15% KCl to remove residual blood and processed identically with kidneys. Liver and kidney were homogenized in 5 vol of ice-cold 0.1 mol/L potassium phosphate buffer (pH 7.4), and the homogenate was spun at 13000*g* for 15 min at 4 °C. The supernatant was then centrifuged at 105000*g* for 60 min at 4 °C and cytosols were stored at −80 °C for subsequent assay of GSHPx activity. Glutathione peroxidase activity was measured according to the method of Wendel (*21*) using 0.2 mmol/L hydrogen peroxide as the substrate and including 1.0 mmol/L sodium azide to inhibit catalase, so that only Se-dependent GSHPx activity was measured. Enzyme activity is expressed as micromoles of substrate hydrolyzed per minute per milligram of protein (abbreviated μU/mg of protein). Protein concentration was determined using the method of Smith et al. (*22*) and with bovine serum albumin as a standard. Blood was withdrawn by cardiac puncture with heparinized syringes for the determination of plasma and red blood cells (RBC) GSHPx activity. The obtained RBC were washed with saline and burst in 9 volumes of hypotonic buffer (5 mmol/L sodium phosphate buffer, pH 7.0). Feces were collected from each rat during the five last days of the repletion period for intestinal absorption measurement.

**Estimation of Se Bioavailability.** The bioavailability of Se from SeSp, pellet, and retentate was evaluated by using sodium selenite refed rats as reference Se. The deposition of Se and the increase in GSHPx activity in different tissues were used as the response to time of repletion (T). Because this response $R$ can be described by the equation $R = mT + k$, the relative bioavailability of Se from the three sources of algal Se was estimated by a slope ratio technique, which compares the slope of time−response plots observed for SeSp, pellet, and retentate to the slope observed for sodium selenite (Se-repleted rats).

**Absorption Spectra Measurements.** Spectroscopic measurements were performed by recording the absorption spectra of retentate and purified phycocyanin in the region of 240−750 nm using a Shimadzu UV-160 A spectrophotometer.

**Statistical Analyses.** Data are shown as the means ± SEM of five measurements per group. Statistical analysis of the data was carried out using Stat View IV software (Abacus Concepts, Berkeley, CA) by two-way ANOVA with Se source (sodium selenite, spirulina, pellet, and retentate) and day of repletion as main effects, followed by a Bonferroni/Dunn multiple comparison. Differences were considered to be significantly different at $P < 0.05$.

## RESULTS

The concentration of selenium in the fortified algae was 223 ± 9 μg/g. The speciation of this selenium has been previously reported (*15*). The total Se content in all three fractions (**Figure 1**) corresponds to the 96.6% of Se found in the starting material. Most of the selenium (∼79%) is present on the pellet. The remaining Se is equally distributed among high (retentate, 11.5%) and low molecular (ultrafiltrate, 9.5%) mass fractions (cutoff at 30 kDa).

**Characteristics of the Pellet (Figure 2).** Hot water extraction was aimed at the recovery of water soluble low molecular mass selenium species. The anion- and cation-exchange chromatograms (not shown) exhibited the presence of at least two species, although the size exclusion chromatography profile suggested the presence of more selenocompounds. Driselase enzymatic extraction allowed liberation of species associated with the cell wall polysaccharides: a selenoprotein and selenomethionine are present. Water insoluble selenoproteins were recovered by SDS solubilization. The size exclusion chromatogram shows the presence of a very intense sharp signal at the exclusion volume of the column ($M_r$ > 15 kDa). Anion- and cation-exchange chromatograms (not shown) had very low intensity, which suggests that the high molecular mass compounds present were not able to pass through the analytical columns; however, a low-intensity peak corresponding to SeMet can be seen. Enzymolysis with lipase and Pronase aimed at the release and digestion of residual selenoproteins resulting in low molecular mass compounds. The size exclusion chromatogram of P consists of a sharp signal eluting at the exclusion volume of the column ($M_r$ > 15 kDa) followed by a group of two poorly resolved peaks in the low molecular mass region. The anion-exchange chromatogram suggests also the presence of SeMet, but it was not confirmed by cation-exchange chromatography.

**Characteristics of the Retentate.** The presence of at least one species of highly anionic character was detected. Anion- and cation-exchange chromatography (not shown) do not allow for the positive identification of the species present.

**Absorption of Se from Spirulina and Subfractions.** Due to the pair-feeding technique, the dietary groups had identical food intake either at 42 days or at 7, 28, and 56 days of Se repletion. Therefore, Se intakes averaged 1.40 μg/day in all groups. Apparent absorption of Se was significantly less from SeSp (82%) than from pellet (86%), retentate (87%), and sodium selenite (89%), which did not differ (**Figure 3**).

**Recovery of Tissue Se and GSHPx Activity.** Selenium repletion in both the liver and kidney is shown in parts A and B of **Figure 4**, respectively: in these organs the restoration of Se was significantly higher in rats fed retentate (111 and 107% of controls, respectively) or sodium selenite (109 and 101% of controls, respectively) than in controls after 56 days. SeSp

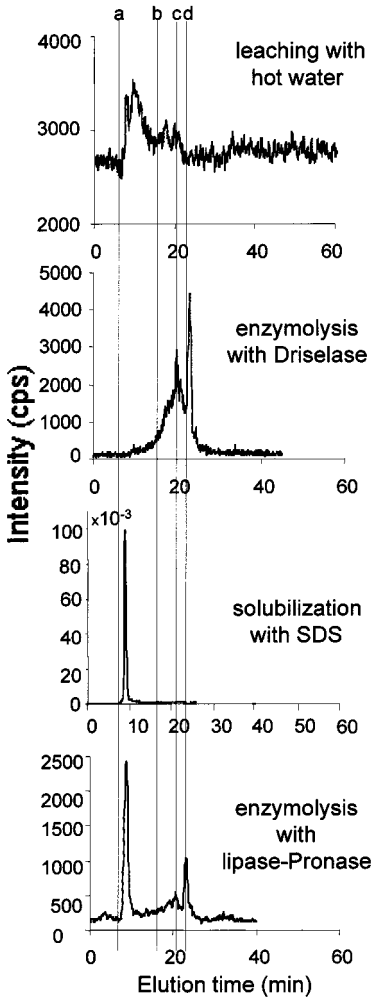


**Figure 2.** Speciation of selenium in the fraction "pellet" by size exclusion HPLC-ICP-MS after sequential extraction. Extractions were with hot water, Driselase solution (mixture of laminarinase, xylanase, and cellulase), SDS solution, and lipase–Pronase solution. Dotted lines mark the exclusion volume (a) and elution volumes of Se(VI) (b), Se(IV) (c), and selenomethionine (d) standards.

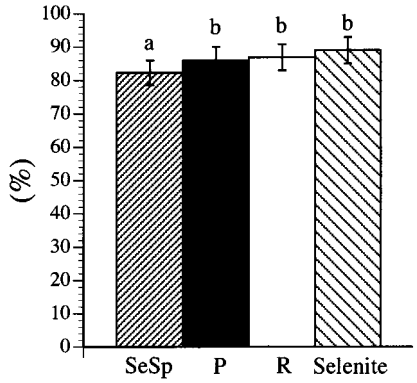


**Figure 3.** Percentage of dietary selenium absorbed by Se-deficient rats after the 56 days of repletion with sodium selenite, Se-rich spirulina (SeSp), pellet (P), or retentate (R). Values are means ± SEM, $n = 5$. Bars with different index letters differ, $P < 0.05$.

cannot normalize liver (52%) and kidney (82%) selenium. Although retentate and spirulina at a lesser extent continued to increase Se in both organs throughout the repletion period, pellet was unable to reach such an efficacy.

Rats fed the TY Se-deficient basal diet for 42 days had only 6.6% of the liver cytosol GSHPx activity of the control animals

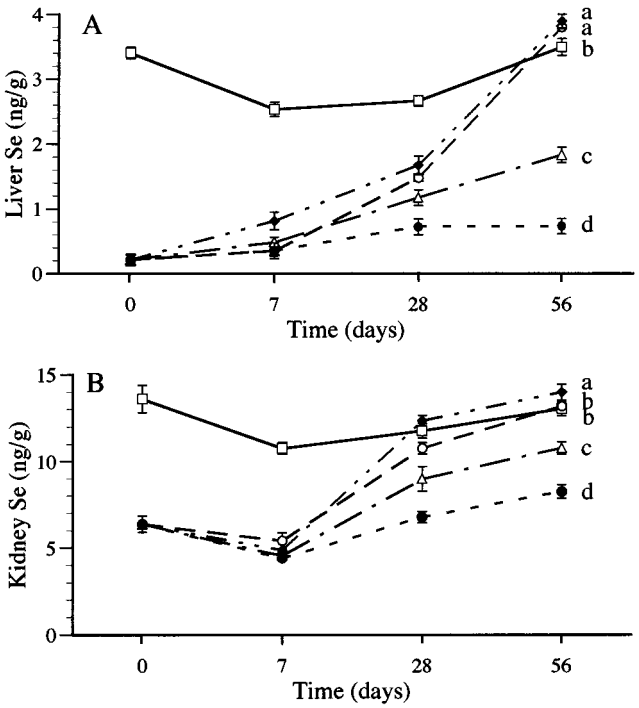


**Figure 4.** Effect of source of Se on the restoration of Se concentration in the tissue of rats fed a low-Se diet for 6 weeks. Rats fed a torula yeast diet for 6 weeks were re-fed a diet containing 0.075 mg of Se/kg with Se supplied as fortified sodium selenite (○), spirulina (△), or spirulina subfractions [pellet (●) and retentate (◆)]. Control animals (□) were fed a diet containing 0.075 mg of Se/kg (Se as selenate) for the entire experiment. Values are the mean of five animals ± SEM. Repletion of Se is shown for liver (A) and kidney (B).

(**Figure 5A**). During the Se repletion period liver GSHPx activity recovered rapidly in retentate and sodium selenite fed groups of animals (115 and 103% of controls, respectively). The rats fed on spirulina had a final recovery of GSHPx activity 2.3-fold lower than with retentate, and final recovery was 50% of controls. Pellet-fed animals showed a significantly lower activity than the other groups during the repletion period (24% of controls). These repletion levels were closely related to repletion of Se concentration in this tissue ($r = 0.95$, 1.14, 1.03, and 0.95 for SeSp, P, R, and sodium selenite, respectively). Correlation coefficients were lower in kidney than in liver ($r = 0.69$, 0.55, 0.88, and 0.86 for SeSp, P, R, and sodium selenite, respectively); kidney cytosol GSHPx activity was not fully recovered by any form of Se (**Figure 5B**), and repletion ranged from 35% of control for pellet to 87% for sodium selenite and 95% for retentate. At the end of the 56 day period, red blood cell GSHPx activity (**Figure 5C**) was not restored in any of the groups; feeding retentate allowed, however, the level to reach 96.5% of controls. With regard to plasma (**Figure 5D**), only retentate allowed fully replenished GSHPx activity (117% of controls), whereas that in the sodium selenite fed group was 84% of controls.

The results of regression analyses between the repletion time and either the tissue Se concentration or GSHPx activities are shown in **Table 2**. The relative bioavailability of Se in SeSp, pellet, and retentate is summarized in **Table 3**. Data from **Figures 4** and **5** are confirmed by those in **Table 3**. Retentate exhibited a very good Se bioavailability (>100%) except for liver Se. On the other hand, Se was poorly available in whole spirulina and pellet.

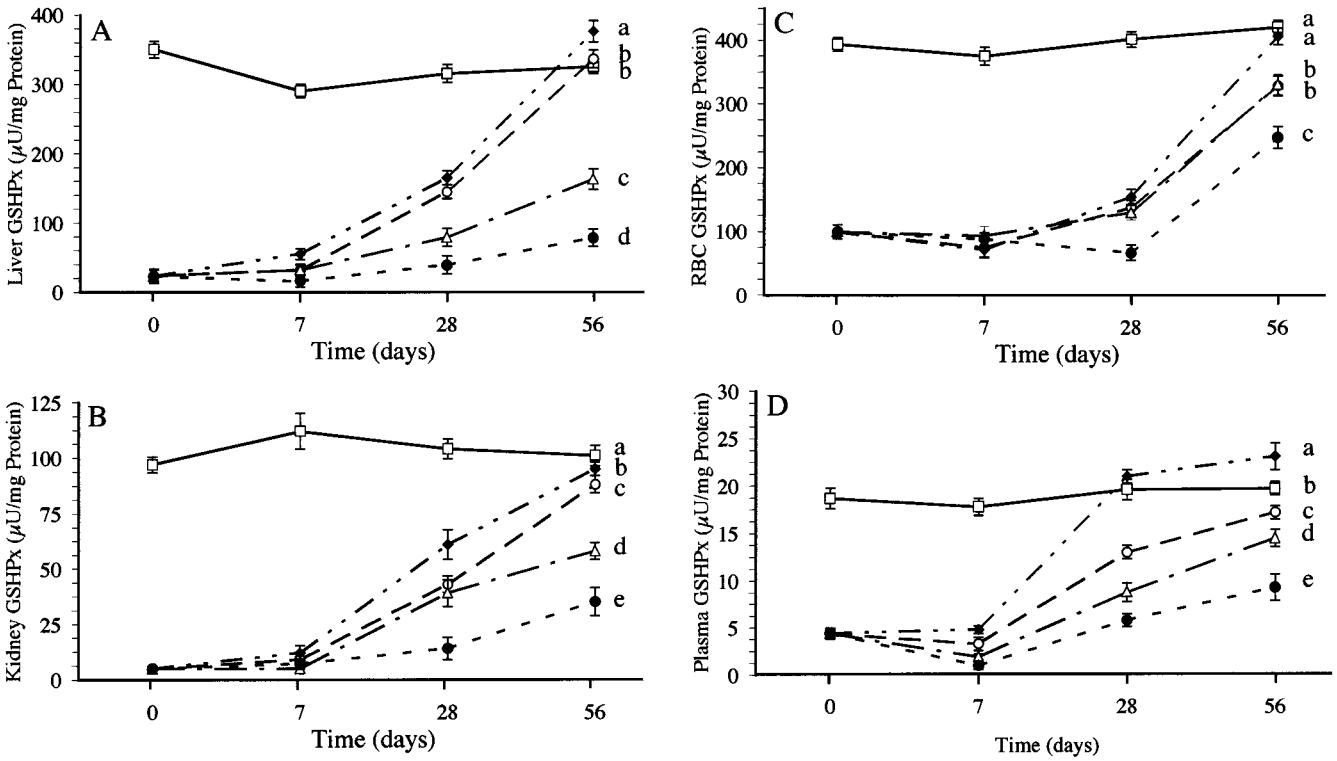


**Figure 5.** Effect of source of Se on the restoration of glutathione peroxidase in tissues. See **Figure 4** for description of experimental methods and legend. Repletion of GSHPx activity is shown for liver cytosol (A), kidney cytosol (B), red blood cells (C), and plasma (D).

**Table 2.** Regression Analyses between Tissue Selenium or GSHPx Activities and Time in Se-Repleted Rats with either Sodium Selenite, Selenium-Rich Spirulina (SeSp), Pellet (P), or Retentate (R)[a]

| dependent variable | slope (*m*) | | | | constant (*k*) | | | | correlation coefficient ($R^2$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | selenite | SeSp | P | R | selenite | SeSp | P | R | selenite | SeSp | P | R |
| Se content | | | | | | | | | | | | |
| liver | 0.071 | 0.027 | 0.007 | 0.063 | 0.28 | 0.33 | 0.37 | 0.99 | 0.98 | 0.99 | 0.83 | 0.98 |
| kidney | 0.146 | 0.123 | 0.070 | 0.164 | 5.47 | 4.35 | 4,14 | 4.35 | 0.96 | 0.95 | 0.97 | 0.98 |
| GSHPx activity | | | | | | | | | | | | |
| liver | 6.237 | 2.700 | 1.270 | 6.600 | –18.20 | 9.24 | 5.74 | –4.51 | 0.99 | 0.99 | 0.99 | 0.99 |
| kidney | 1.612 | 1.059 | 0.580 | 1.612 | –2.22 | –1.85 | 1.04 | –0.43 | 1.00 | 0.97 | 0.98 | 0.98 |
| erythrocytes | 5.272 | 4.963 | 3.420 | 5.272 | 19.74 | 33.44 | 29.83 | 34.14 | 0.98 | 0.96 | 0.85 | 0.98 |
| plasma | 0.263 | 0.271 | 0.166 | 0.263 | 2.96 | 0.44 | 0.30 | 0.44 | 0.94 | 0.99 | 0.98 | 0.97 |

[a] Regression was fitted to the equation $R = mT + k$, where $R$ represented tissue Se content or GSHPx activity in Se-repleted rats with either sodium selenite, selenium-rich spirulina, pellet, or retentate. Units of dependent variables were the same as in **Figures 4** and **5**.

**Table 3.** Relative Bioavailability of Selenium Contained in Se-Rich Spirulina, Pellet (P), and Retentate (R)[a]

| parameter | spirulina | P | R |
|---|---|---|---|
| Se content | | | |
| liver | 38 | 98 | 89 |
| kidney | 84 | 48 | 112 |
| GSHPx activity | | | |
| liver | 43 | 20 | 106 |
| kidney | 66 | 36 | 103 |
| erythrocytes | 94 | 65 | 131 |
| plasma | 103 | 63 | 133 |

[a] Relative biological availability was estimated by using the slope of the regression line described in **Table 3**; (slope of SeSp or P or R)/(slope of sodium selenite) × 100.

## DISCUSSION

The minimum dietary Se concentration that will meet animal requirements has been reported to lie in the range of 50−100 μg of Se/kg (*23*). In this study, we used 75 μg of Se/kg in control and Se repletion diets. This suboptimal level was chosen to generate nonplateauing GSHPx activities (*23*) because the use of 100 μg of Se/kg most likely would mask any metabolic differences that might have been detected with a level lower than the dietary Se requirement.

The replacement of the inorganic Se compounds as feed additives can be made by SeMet (*25*) or nutritional sources. Yeast growing in Se-enriched media was reported to have beneficial effects (*26*). In response to the need for Se to support human health, selenium-enriched foods have been developed in recent years: high-Se Brussels sprouts (*27*), high-Se celery (*28*), and high-Se *Brassica* vegetables (*29*). High-Se garlic was shown to have a great protective effect against mammary cancer (*30*) and high-Se broccoli against mammary and colon cancer (*16*, *31*). In the search for an economical source of organic nutritional forms of Se, we attempted to increase the normally low Se content of spirulina by growing it in Se-enriched medium (*15*): although selenium from Se-enriched spirulina is biologically useful, its bioavailability was not superior to that of the inorganic Se salt (sodium selenite) or of SeMet. This may be

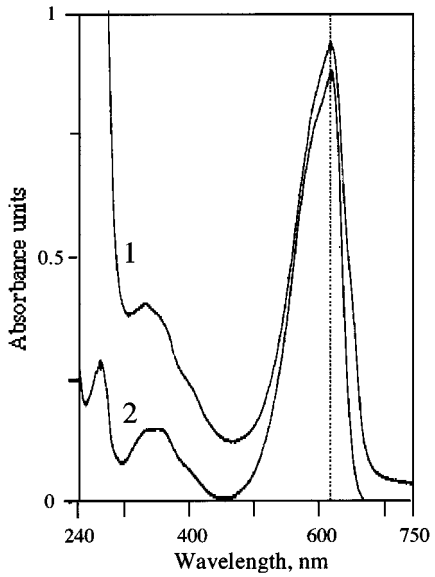


**Figure 6.** Absorption spectra of retentate (1) and purified phycocyanin (2). Measurements were made in the region of 240–750 nm. Dotted line marks the 620 nm wavelength.

due in part to a reduced alga degradation in the digestive tract and to different metabolic pathways used by Se of the different forms utilized in this study. Therefore, we have prepared spirulina subfractions, and one of the main purposes of the present study was to assess the bioavailability of Se in each fraction. Generally, the apparent absorption of the organic Se compounds in foods is deemed to be good (~70−95%) (*32*). Our results are consistent with these values.

Spirulina is an unbranched, helicoidal, filamentous blue-green alga or cyanobacterium, which has long been consumed by humans. Besides proteins, spirulina biomass contains lipids, carbohydrates, and photosynthetic pigments (chlorophyll, carotenoids, and phycocyanin). The latter is responsible for the characteristic blue-green color of spirulina and resembles biliary pigments. The spirulina biomass fractionation process aimed at the separation of water soluble proteins is well-known (*33*) and was used in this study with a slight modification by the addition of the ultrafiltration step. The hydrosoluble fraction obtained after removal of the pellet is an intensely blue-colored fraction because phycocyanin is the main component. This was confirmed by the UV−visible spectrum of retentate in comparison with that of purified phycocyanin (**Figure 6**). Phycocyanins are trimeric phycobiliproteins (.~110 kDa) that can be in the form of monomers at pH 6−8 and at low ionic strength. Monomers themselves are made up of two subunits (~17−18 kDa range). The fractionation process used produces a pellet rich in proteins and lipids and a retentate (mainly phycocyanins).

Elsewhere, consumption of spirulina has substantial benefit and nutritional interest (*18*) unrelated to selenium; the fraction called "retentate" (i.e., phycocyanin) exerts scavenging action against reactive oxygen species such as $OH^{\bullet}$ and $RO^{\bullet}$ radicals and produces a concentration-dependent decrease in lipid peroxidation induced by $Fe^{2+}$−ascorbic acid in rat liver microsomes (*34*). It has also been reported that phycocyanin is an antioxidant protector for human erythrocytes against lysis by peroxyl radicals (*35*). Spirulina grown in the presence of Se accumulates substantial amounts of Se (*17*), and issuing fractions contain also Se. Therefore, it is possible that the health benefits of spirulina and more particularly of retentate may be improved by Se fortification. At the start of this study, nothing was known of how Se from spirulina and its subfractions is absorbed, distributed, and metabolized in the body; this was another purpose of this work, in comparison to a form of Se that has been more often studied, sodium selenite.

Selenium from retentate was superior to that from other fractions and from SeSp in restoring tissular GSHPx activity and Se concentration. In fact, we have previously reported that the majority of Se in spirulina was present in the form of selenoproteins (*15*). Moreover, phycocyanin represents ~25% of the cellular protein, highly digestible and with high biological value (*36*): this could represent one explanation for why Se from retentate is so highly available. Its relative bioavailability that is >100% (except for the liver) is just written off to experimental error. This means that Se from retentate was as effective as the inorganic form, sodium selenite. As reported by Finley (*37*) one criterion to be beneficial is that the supplemental form of Se must be relatively nontoxic, meaning that it should not accumulate excessively in the body, but when dietary Se is limiting, it should also be able to replenish the Se pool used for selenoprotein synthesis. Our results showed that in our experimental conditions the retentate globally meets the first criterion for a good supplemental form of Se, despite a bioavailability slightly >100% (kidney Se). It meets the second one without reservation.

The slope−ratio technique used in this study is a reasonable procedure (here the correlation coefficients of the regressions ranged from 0.83 to 0.99) for the assessment of bioavailability of Se from different sources as reported by Yoshida et al. (*38*). The authors measured Se bioavailability from selenized yeasts that exceeded that for selenite, ranging from 105 to 197% according to the measured parameters; for comparison, Se retentate exhibited values ranging from 83 to 130%. Other observations using the slope−ratio analysis reported that the relative bioavailability of Se from yeast was >100% (*39*); despite this, selenized yeasts were reported to be useful as an Se source to improve human Se status. Elsewhere, in our study, there is a consistency between tissue Se concentrations and GSHPx activities; moreover, we have shown that Se from retentate was at least as efficient as that from sodium selenite, although it did not have the same chemical form (mainly incorporated into phycocyanin in retentate). On the other hand, in the pellet, Se is in a form actually ill-defined and poorly efficient. Therefore, it can be concluded that retentate issuing from Se-rich spirulina fractionation may be a valuable Se source of high availability in food supplementation within the range of recommended daily Se intake. Further investigations (i) to identify the Se form of high molecular mass species for which no analytical standards are available and (ii) to evaluate the potential biological activities of retentate need to be conducted before a recommendation of this fraction as an Se supplement.

## ACKNOWLEDGMENT

We gratefully acknowledge Dr. Christian Aymard (CIRAD, Montpellier, France) for his helpful criticism in statistical analysis.

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**Received for review December 11, 2001. Revised manuscript received March 27, 2002. Accepted March 27, 2002.**

JF011646T