

## Research Article

# Improvement of Physiological Characteristic of Selenium-Enriched *Candida utilis* with Amino Acids Addition

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The effects of amino acids addition on cell growth, glutathione biosynthesis, glutathione distribution, and the intracellular oxidation-reduction environment of *Candida utilis* SZU 07-01 during selenium enrichment were investigated in this study. Most amino acids under appropriate concentrations have positive effects on cell growth of the yeast strain, except for phenylalanine and proline, compared with the control without amino acid addition. The bioconversion of selenite to organic selenium induced the reduction of glutathione synthesis and intracellular distribution of glutathione. However, amino acids including cysteine, glutamine, glutamic acid, isoleucine, leucine, and tyrosine could effectively promote the selenium-enriched yeast to elevate glutathione production, especially increasing the intracellular glutathione content. Moreover, addition of these six different amino acids apparently decreased malondialdehyde concentration and recovered the normal intracellular redox environment of the selenium-enriched *C. utilis* SZU 07-01. The improvement of physiological characteristic of the selenium-enriched yeast by increasing intracellular glutathione content and lowering malondialdehyde content will undoubtedly help to widen application of selenium-enriched yeast as food or feed additives.

## 1. Introduction

Selenium is an essential nutritional trace element for many mammalian species including human beings owing to its critical role involved in cell metabolism [1]. Selenium is also an essential component of the active site of selenoenzyme glutathione peroxidase [2]. This enzyme, together with catalase and superoxide dismutase, protects cells against damages caused by free radicals and lipid peroxides [3]. Selenium is now being widely studied for its cancer chemopreventive activity and antioxidative property [4], and it also has a profound effect on survival of HIV-infected patients [5]. In mammals, selenium deficiency has been associated with human diseases including muscular, neurological, and immune disorders, and also with increased cancer incidence and mortality [1, 6].

The deficiency of selenium in the diet is compensated for supplementation [7]. However, selenium can be either essential or toxic depending on its chemical form and concentration, and its safety and efficacy may also be markedly dynamic because of differential metabolic

processing by different organs. It is generally believed that the ingestion of organic selenium compounds is better and safer than that contained inorganic selenium [8]. Many organic and inorganic selenium materials have been investigated as selenium supplements. Among them, supplementation by using selenium-enriched microorganisms has received much attention in recent years [9]. Researchers have found that yeast is a good carrier for selenium biotransformation. Under appropriate conditions, yeast is capable of producing biomass with high protein content and meanwhile accumulating large amount of trace elements such as selenium, and transforming inorganic selenium (low bioavailability, potentially toxic) into organic form (safer and highly bioactive), mostly in the form of selenomethionine [8].

In June 2000, selenium-enriched yeast was approved by FDA as a source for feed-supplemented organic selenium for chickens [10], which can also provide antioxidant protection at a level greater than inorganic selenium for chickens and other livestock [11]. Organic selenium from selenium-enriched yeast has been applied to the improvement of meat quality [12], growth of feathers [13], and positive influence

of thyroxine conversion to tri-iodothyronine and passive immunity of newborn lambs [14]. Hence, the commercial demand for selenium-enriched yeast in the future will increase gradually.

Glutathione, a low-molecular thiol compound found in most plants, animals and microorganisms, fulfills its roles in many cellular processes including the protection of DNA, proteins, and other biomolecules against oxidative damage caused by reactive oxygen species [15]. Glutathione has elicited many interests in the fields of medical treatment, health care, therapeutics, sports nutrition, feed additive and cosmetics industry [16]. Glutathione can be synthesized by chemical method, enzymatic reaction, and microorganism fermentation [17]. Among these methods, yeast fermentation is more efficient and practical. Microorganisms such as *Saccharomyces cerevisiae* and *Candida utilis* are commonly used for fermentative production of glutathione [18, 19].

Based on the physiological function of glutathione on living organisms, the application of selenium-enriched yeast will be greatly expanded if the yeast contained glutathione intracellularly. However, most previous works on selenium-enriched yeast were carried out by *S. cerevisiae*, in which low yield of biomass and conversion rate of inorganic selenium were major obstacles for successful commercialization of this microorganism in the health food and feed industry [9]. So, we paid close attention to screening other industrial used yeast of *C. utilis* for selenium-enriched yeast preparation, and have found that *C. utilis* has a higher conversion rate from inorganic selenium to organic form, together with less ethanol formation during the cultivation [20]. Before our studies, few reports were focused on this aspect to date, nor with the quality improvement of selenium-enriched *C. utilis*. Moreover, the intracellular glutathione content in yeast cells was rarely explored although total selenium content was more concerned. Here, we investigated the effects of amino acids addition on the preparation of selenium-enriched *C. utilis*, with special emphasis on the enhancement of intracellular glutathione content of the selenium-enriched yeast in order to improve its application as food or feed additives.

## 2. Materials and Methods

**2.1. Yeast Strain and Medium.** *C. utilis* SZU 07-01, a strain which can produce glutathione intracellularly together with great ability to absorb selenium, was used in this study. The yeast strain was maintained at 4°C by being monthly subcultivated on the slant with seed medium (20 g/L glucose, 20 g/L peptone and 10 g/L yeast extract, pH 6.0) and 20 g/L agar.

**2.2. Culture Method.** The seeds were prepared by transferring colonies from a fresh agar slant into 500 mL Erlenmeyer flasks containing 50 mL seed medium, and incubated at 30°C for 20 hours on a rotary shaker with an agitation rate of 200 rpm. Then the culture was inoculated at 10% (v/v) into the fermentation broth. Unless stated otherwise, the medium for fermentation contained glucose (30 g L<sup>-1</sup>),

ammonium sulfate (8 g L<sup>-1</sup>), KH<sub>2</sub>PO<sub>4</sub> (3 g L<sup>-1</sup>), MgSO<sub>4</sub> (0.25 g L<sup>-1</sup>), Na<sub>2</sub>SeO<sub>3</sub> (0.01 g L<sup>-1</sup>), with an initial pH of 5.5. The preparation of Se-enriched yeast was carried out in 500 mL flasks containing 40 mL fermentation medium, and the culture conditions were fixed at 27°C, 200 rpm and 30 h, respectively [20]. The medium was steam-sterilized except for Na<sub>2</sub>SeO<sub>3</sub>, which was microfiltered by a Sartorius membrane with the pore size of 0.20 μm.

**2.3. Amino Acids Addition in Flasks.** Each amino acid was added to the fermentation broth at the beginning of cultivation (0 hour), and the addition concentration of each amino acid shifted from 2 mmol L<sup>-1</sup> to 16 mmol L<sup>-1</sup>. The concentrated amino acid solution was also microfiltered by a Sartorius membrane with the pore size of 0.20 μm. All amino acids added in this work are L-isomers, hence we omit "L-" for each amino acid in the following text.

**2.4. Analytical Methods.** A culture broth of 25 mL was centrifuged at 8,000 × g for 10 minutes, and after washed twice with distilled water, the wet cells were dried at 70°C to a constant weight for dry cell weight (DCW) determination. Glutathione was extracted from the wet cells by 40% (v/v) ethanol solution at 30°C for 2 hours, then centrifuged at 8,000 × g for 15 minutes, the supernatant was used for glutathione assay. Glutathione concentration was determined using DTNB and glutathione reductase according to the method described by Tietze [21]. The intracellular glutathione content (IGC) of selenium-enriched yeast was defined as follows:

Intracellular glutathione content/%

$$= \frac{\text{glutathione concentration}/(\text{mgL}^{-1})}{\text{DCW}(\text{gL}^{-1}) \times 10} \times 100\% \quad (1)$$

Wet cells from a 5 mL culture broth were washed twice with deionized water, after centrifuged at 8,000 × g for 15 minutes, the cells were digested in 10 mL concentrated nitric acid and perchloric acid (v:v = 4:1) at 100°C for 10 minutes. To avoid the volatilization loss of selenium, one reflux equipment was employed during the digestion process. The digested samples were diluted to a final volume of 50 mL with 0.1 mol L<sup>-1</sup> HCl, filtered with a Sartorius membrane with the pore size of 0.20 μm, and the concentration of total selenium was determined with a catalytic spectrophotometry followed with the method previously described by Hao and Teng [22].

Wet cells from a 10 mL fermentation broth were washed three times with deionized water, then mixed with 5 mL ultrapure water and boiled at 100°C for 1 hour, the supernatant was assayed for the intracellular inorganic selenium. Organic selenium was calculated from the difference between the total selenium and inorganic selenium.

Glucose was determined by the DNS spectrometric method [23], and malondialdehyde (MDA) was determined by TBA assay [24]. The results shown from the flasks represent an average of three parallel experiments.

TABLE 1: Comparison of parameters on the batch process with and without selenium enrichment.

Parameters	Without selenium enrichment	With selenium enrichment
Dry cell weight ( $\text{g L}^{-1}$ )	$11.26 \pm 0.08$	$10.14 \pm 0.06$
Intracellular glutathione content (%)	$0.89 \pm 0.03$	$0.14 \pm 0.03$
Extracellular glutathione ( $\text{mg L}^{-1}$ )	$41.3 \pm 2.7$	$60.7 \pm 3.2$
Total glutathione ( $\text{mg L}^{-1}$ )	$142.0 \pm 5.4$	$74.6 \pm 4.6$
Yield of selenium biotransformation (%)	$0 \pm 0.0$	$90.2 \pm 0.6$

TABLE 2: Effects of amino acids addition on cell growth of selenium-enriched yeast.

Amino acid ( $\text{mmol L}^{-1}$ )	Dry cell weight ( $\text{g L}^{-1}$ )						Maximum ratio increased (%)
	0	2	4	6	8	10	
Asp	$10.76 \pm 0.04$	$10.52 \pm 0.03$	$11.36 \pm 0.02$	$11.56 \pm 0.02$	$12.48 \pm 0.03$	$12.25 \pm 0.01$	16.0
Leu	$9.53 \pm 0.08$	$10.30 \pm 0.04$	$10.43 \pm 0.03$	$10.63 \pm 0.08$	$10.90 \pm 0.03$	$10.73 \pm 0.08$	14.4
Thr	$10.35 \pm 0.00$	$10.95 \pm 0.05$	$10.95 \pm 0.05$	$11.55 \pm 0.05$	$11.43 \pm 0.08$	$11.70 \pm 0.05$	13.0
Val	$9.70 \pm 0.05$	$10.00 \pm 0.04$	$10.25 \pm 0.02$	$10.55 \pm 0.05$	$10.95 \pm 0.04$	$10.25 \pm 0.01$	12.9
Lys	$10.23 \pm 0.03$	$10.10 \pm 0.05$	$10.58 \pm 0.08$	$10.75 \pm 0.05$	$11.13 \pm 0.03$	$11.48 \pm 0.08$	12.2
Arg	$9.60 \pm 0.02$	$9.68 \pm 0.08$	$9.90 \pm 0.05$	$9.95 \pm 0.02$	$10.23 \pm 0.08$	$10.65 \pm 0.00$	10.9
Ser	$10.76 \pm 0.04$	$10.98 \pm 0.03$	$11.15 \pm 0.03$	$11.40 \pm 0.03$	$11.73 \pm 0.03$	$11.80 \pm 0.05$	9.7
Ile	$9.53 \pm 0.08$	$9.95 \pm 0.05$	$10.03 \pm 0.08$	$10.13 \pm 0.03$	$10.23 \pm 0.03$	$10.43 \pm 0.08$	9.4
Gln	$9.90 \pm 0.05$	$9.93 \pm 0.03$	$10.00 \pm 0.05$	$10.43 \pm 0.03$	$10.80 \pm 0.04$	$10.73 \pm 0.03$	9.1
His	$9.90 \pm 0.06$	$10.20 \pm 0.06$	$10.49 \pm 0.07$	$10.49 \pm 0.03$	$10.72 \pm 0.03$	$10.69 \pm 0.07$	8.3
Glu	$10.38 \pm 0.00$	$10.07 \pm 0.00$	$10.31 \pm 0.01$	$10.44 \pm 0.02$	$10.94 \pm 0.07$	$11.14 \pm 0.01$	7.3
Ala	$10.76 \pm 0.04$	$10.36 \pm 0.03$	$10.24 \pm 0.02$	$10.80 \pm 0.03$	$10.88 \pm 0.05$	$11.40 \pm 0.08$	5.9
Tyr	$9.60 \pm 0.02$	$9.70 \pm 0.10$	$9.70 \pm 0.10$	$9.65 \pm 0.02$	$9.85 \pm 0.05$	$10.00 \pm 0.05$	4.2
Cys	$9.90 \pm 0.06$	$9.48 \pm 0.02$	$9.63 \pm 0.07$	$10.30 \pm 0.03$	$10.06 \pm 0.06$	$9.54 \pm 0.01$	4.0
Asn	$9.90 \pm 0.5$	$9.43 \pm 0.03$	$9.80 \pm 0.00$	$9.85 \pm 0.02$	$10.00 \pm 0.05$	$10.28 \pm 0.03$	3.8
Met	$10.98 \pm 0.00$	$11.03 \pm 0.03$	$11.08 \pm 0.04$	$11.13 \pm 0.02$	$11.22 \pm 0.03$	$11.32 \pm 0.04$	3.1
Try	$10.23 \pm 0.03$	$9.83 \pm 0.08$	$10.13 \pm 0.03$	$10.25 \pm 0.02$	$10.53 \pm 0.03$	$10.30 \pm 0.01$	2.9
Gly	$10.38 \pm 0.00$	$9.98 \pm 0.05$	$10.14 \pm 0.02$	$10.47 \pm 0.08$	$10.54 \pm 0.08$	$10.63 \pm 0.02$	2.4
Pro	$10.23 \pm 0.03$	$9.93 \pm 0.03$	$8.08 \pm 0.08$	$8.58 \pm 0.03$	$9.05 \pm 0.05$	$8.98 \pm 0.08$	-2.9
Phe	$10.23 \pm 0.03$	$9.65 \pm 0.02$	$8.80 \pm 0.00$	$8.90 \pm 0.01$	$9.15 \pm 0.00$	$9.03 \pm 0.03$	-5.7

### 3. Results

**3.1. Effect of Selenium Enrichment on Batch Culture of *C. utilis* SZU 07-01.** The preparation of selenium-enriched yeast by cultivation of *C. utilis* SZU 07-01 was carried out in flasks, followed with the optimal nutrimental and environmental conditions as described in our previous study [20]. Under this circumstance, more than 90% of inorganic selenium was assimilated by the yeast and transformed into organic selenium. However, several parameters altered dramatically compared with the process without selenium enrichment (Table 1). For instance, DCW, total glutathione and intracellular glutathione content decreased by 10%, 47% and 84%, respectively. It is well known that higher intracellular glutathione content can boost the quality and application area of selenium-enriched *C. utilis* in food and feed industry [16], since it can fulfill organic selenium supplement together with the improvement of immunity of animals. Therefore, we should take measures such as amino

acids addition to improve total glutathione and intracellular glutathione content of the Se-yeast.

**3.2. Amino Acid Addition Boosted the Cell Growth of Selenium-Enriched Yeast.** Amino acids, the basic units of protein and growth factor, are essential nutrient elements for the life cycle of yeast. Although *C. utilis* SZU 07-01 can grow well on a synthetic medium just contained glucose, ammonium and ions, it is also shown that the addition of some amino acids is beneficial to cell growth and metabolism of the yeast [25]. Whether amino acids were added or not, glucose in the culture medium was completely consumed, followed that glutathione was biosynthesized intracellularly and selenium was assimilated. As is shown in Table 2, almost all the amino acids added with appropriate concentration have positive effect on cell growth of selenium-enriched *C. utilis* SZU 07-01 except for Phe and Pro, of which Ala, Gln, Glu, His, Ile and Ser can improve the biomass by 5 ~ 10%, Arg, Leu, Lys,

TABLE 3: Effects of amino acids addition on glutathione biosynthesis of selenium-enriched yeast.

Amino acid (mmol L <sup>-1</sup> )	Glutathione (mg L <sup>-1</sup> )						Maximum ratio increased (%)
	0	2	4	6	8	10	
Cys	66.2 ± 1.6	74.8 ± 0.4	79.5 ± 2.6	92.8 ± 0.3	109.2 ± 2.2	134.2 ± 1.7	102.7
Ala	73.1 ± 2.5	94.8 ± 1.7	130.4 ± 2.5	68.8 ± 0.6	78.9 ± 1.2	63.8 ± 2.1	78.4
Asp	73.1 ± 2.5	47.6 ± 0.6	97.6 ± 1.6	121.1 ± 2.1	58.7 ± 0.8	75.7 ± 1.2	65.7
Glu	82.0 ± 0.7	69.7 ± 0.5	90.1 ± 0.0	105.8 ± 0.3	110.6 ± 4.7	127.2 ± 0.4	55.1
Gln	71.4 ± 1.2	72.6 ± 1.6	71.0 ± 2.4	81.3 ± 0.2	93.5 ± 1.1	106.9 ± 1.3	49.7
Leu	77.0 ± 1.1	73.2 ± 1.2	93.7 ± 0.5	98.8 ± 0.6	109.8 ± 1.7	115.1 ± 2.5	49.5
Met	87.5 ± 1.5	94.5 ± 2.2	101.4 ± 1.6	108.4 ± 1.2	118.6 ± 2.6	130.7 ± 3.4	49.4
Tyr	62.1 ± 1.8	60.9 ± 0.1	73.8 ± 0.3	82.2 ± 0.0	82.5 ± 4.9	91.9 ± 1.4	48.0
Arg	62.1 ± 1.8	70.4 ± 2.6	66.4 ± 2.0	77.0 ± 2.1	86.0 ± 1.6	77.9 ± 2.3	38.5
Ile	77.0 ± 1.1	80.6 ± 1.0	88.1 ± 2.0	93.7 ± 0.3	102.4 ± 1.0	101.3 ± 2.1	33.0
Val	61.4 ± 0.7	69.9 ± 0.4	71.1 ± 1.2	71.3 ± 2.1	76.2 ± 0.6	79.6 ± 2.6	29.6
Try	88.1 ± 1.9	91.3 ± 0.6	95.6 ± 0.3	96.2 ± 4.5	105.0 ± 1.0	111.0 ± 2.7	26.0
His	66.2 ± 1.6	72.7 ± 1.7	76.9 ± 0.3	80.7 ± 2.4	83.2 ± 2.3	82.6 ± 1.2	25.7
Thr	73.5 ± 4.6	82.2 ± 2.6	77.9 ± 2.4	78.7 ± 0.8	84.7 ± 2.2	85.6 ± 3.2	16.5
Ser	73.1 ± 2.5	57.2 ± 1.7	67.6 ± 0.4	74.6 ± 0.4	84.0 ± 0.4	81.0 ± 2.4	14.9
Gly	82.0 ± 0.7	64.5 ± 0.9	70.1 ± 0.4	77.2 ± 1.7	92.5 ± 2.5	90.5 ± 1.3	12.8
Asn	71.4 ± 1.2	63.6 ± 1.1	69.7 ± 0.5	68.3 ± 0.9	71.6 ± 1.8	75.0 ± 2.2	4.7
Phe	88.1 ± 1.9	80.6 ± 2.5	75.0 ± 3.7	78.4 ± 0.3	82.2 ± 2.0	85.2 ± 0.3	-3.3
Lys	78.0 ± 0.8	66.4 ± 1.8	69.7 ± 0.7	67.3 ± 1.4	64.1 ± 0.4	66.8 ± 0.0	-10.6
Pro	78.0 ± 0.8	45.5 ± 1.8	29.7 ± 3.0	21.8 ± 0.9	31.8 ± 1.1	36.2 ± 0.7	-41.7

Thr and Val can increase cell growth by 10 ~ 15%. Specially, DCW of selenium-enriched *C. utilis* SZU 07-01 was elevated by 16% when 8 mmol/L of Asp was added to the medium at the beginning of cultivation.

**3.3. Effect of Amino Acids Addition on Glutathione Biosynthesis during Selenium Enrichment.** Glutathione is biosynthesized with its precursors (glutamic acid, cysteine and glycine) and ATP, hence the addition of precursor amino acids in accordance with appropriate concentration is associated with increased glutathione biosynthesis. However, the assimilation of selenite by yeast cells will also facilitate the excretion of glutathione to the outer broth. The process of glutathione biosynthesis including total glutathione, intracellular glutathione and extracellular glutathione was investigated when the precursors and other amino acids were added during the preparation of selenium-enriched *C. utilis* SZU 07-01. It was found in Table 3 that just adding Lys, Phe and Pro have no positive effect on glutathione synthesis when inorganic selenium was biotransformed into organic forms. Other amino acids can increase glutathione yield if they were added in appropriate concentrations. His, Ser, Thr and Try can increase glutathione synthesis by 10 ~ 30%, Arg, Gln, Ile, Leu, Met and Tyr can elevate glutathione by 30 ~ 50%, Ala, Asp and Glu can boost glutathione yield by more than 50%. Specially, cysteine, the key amino acid during glutathione synthesis, has a prominent ability to improve glutathione by 102.7%.

**3.4. Effect of Amino Acids Addition on the Distribution of Glutathione.** Although many amino acids can boost total

glutathione biosynthesis even in the presence of selenite, the intracellular glutathione content after adding different amino acids varied dramatically. It can be calculated from Table 4 that most of amino acids except Asn and Lys had positive effect on intracellular glutathione content of selenium-enriched *C. utilis* SZU 07-01. Moreover, the tendency of glutathione distribution extracellularly altered opposite to intracellular glutathione content. Among all the amino acids we considered, Cys, Glu, Ile, Leu were helpful in resistance of glutathione leakage to outer broth, and Leu was the most prominent one to promote intracellular glutathione content, together with intracellular distribution of glutathione, when compared with the control without amino acid addition within the process of selenium enrichment by *C. utilis* SZU 07-01.

**3.5. Effect of Amino Acids Addition on the Intracellular Oxidation-Reduction Environment.** During selenium enrichment of *C. utilis* SZU 07-01, more reduced glutathione was excreted to the outer cells and transformed to oxidized form. The decrease in reduced glutathione content may decrease the activities of antioxidant enzymes and so it aggravates the effect of oxidative stress [26]. The intracellular oxidation-reduction environment was represented with the inner concentration of malondialdehyde (MDA) in this study. It was illustrated in Figure 1 that Cys, Gln, Glu, Ile, Leu and Tyr which have significant effects on promoting the intracellular glutathione content and making more glutathione be distributed intracellularly can also apparently reduce MDA concentration and recover the intracellular redox environment of selenium-enriched of *C. utilis* 07-01.

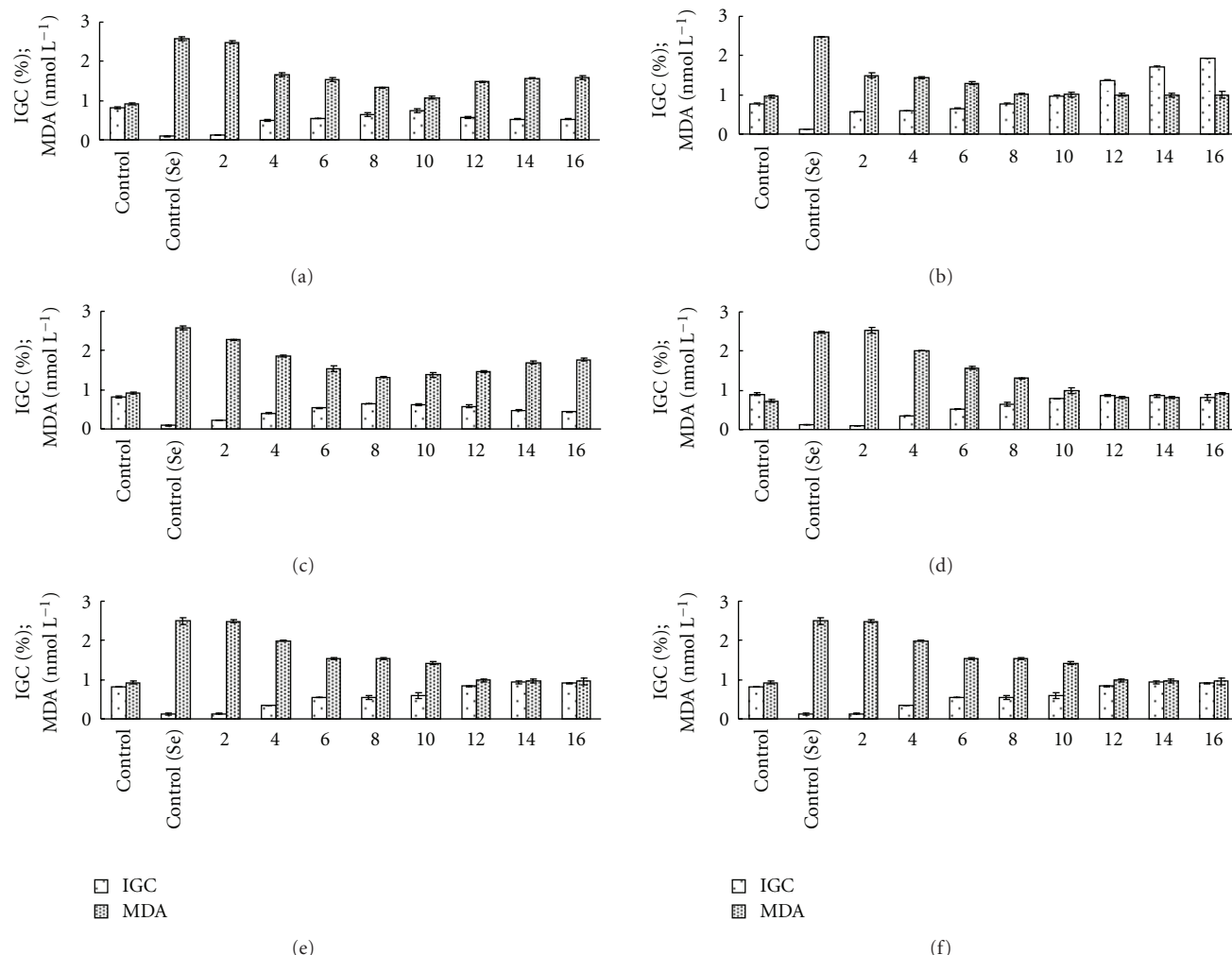


FIGURE 1: Effects of different concentration of amino acids ((a), Leu; (b), Cys; (c), Gln; (d), Glu; (e), Ile; (f), Tyr) addition on intracellular glutathione content and MDA concentration during selenium-enriched *C. utilis* SZU 07-01 preparation. The control represents batch culture without selenium enrichment; the control (Se) represents batch culture with selenium enrichment but without amino acids addition.

The more intracellular content of glutathione was detected, the less concentration of inner MDA was observed.

#### 4. Discussion

More than 90% of selenium assimilated in selenium-enriched yeast involving *C. utilis* existed in the form of selenomethionine [8]. The metabolic pathway from selenomethionine to selenocysteine could provide Se-cysteine for the active site of glutathione peroxidase, which could enhance the enzymatic activity and thus promote the oxidation of glutathione. Moreover, selenium of low valence could be oxidized to higher oxidation state by O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> and other peroxides, which could produce reactive oxygen species (ROS). ROS and selenium of high valence could be reduced further by glutathione. Therefore, with the biotransformation of selenium, the more selenium was absorbed to inner cells, the less intracellular glutathione was reserved and the more ROS was formed. Besides, selenite

could also induce the leakage of glutathione to the outer broth during selenium enrichment [27].

Glutathione is one of the most important compounds in maintaining intracellular thiol status. It performs various functions ranging from cellular metabolism to transport and also protection against oxygen-free radicals [28]. Therefore the reduction of intracellular glutathione content would break the delicate balance between the prooxidant forces and antioxidant defenses which is known as the redox balance. When the antioxidant defenses are overwhelmed by prooxidants, oxidative stress may occur and so aggravate the effects of oxidative stress [26]. Under high oxidative stress, the production and accumulation of lipid peroxidation could be increased due to the increase of ROS. Damage to mitochondria induced by lipid peroxidation can lead to further generation of ROS [29]. In addition, increased lipid peroxidation can lead to the production of MDA that would enhance the formation of free radicals from polyunsaturated fatty acids in cell membranes and cause depletion of glutathione through detoxification by glutathione peroxidase



TABLE 4: The ratio of extracellular glutathione decreased with amino acids addition.

Amino acid (mmol L <sup>-1</sup> )	The ratio of extracellular glutathione (%)						Maximum ratio decreased (%)
	0	2	4	6	8	10	
Cys	80.6 ± 0.3	28.6 ± 0.5	28.8 ± 0.4	28.0 ± 0.3	29.2 ± 0.1	30.9 ± 0.2	65.3
Glu	83.3 ± 0.2	84.6 ± 0.5	61.0 ± 0.0	47.7 ± 0.4	36.0 ± 0.3	31.6 ± 0.1	62.1
Leu	89.1 ± 0.1	82.2 ± 0.3	45.4 ± 0.2	41.9 ± 0.5	36.8 ± 0.4	30.3 ± 0.2	66.0
Ile	89.1 ± 0.1	72.5 ± 0.3	54.1 ± 0.5	41.4 ± 0.2	36.2 ± 0.1	36.8 ± 0.4	59.4
Tyr	80.9 ± 0.4	79.8 ± 0.4	54.3 ± 0.2	35.8 ± 0.1	35.4 ± 0.2	35.3 ± 0.4	56.4
Asp	78.0 ± 0.4	74.1 ± 0.1	41.6 ± 0.3	39.7 ± 0.4	81.9 ± 0.5	82.6 ± 0.3	49.1
Gln	83.9 ± 0.5	80.6 ± 0.3	83.8 ± 0.4	74.3 ± 0.2	68.1 ± 0.1	45.0 ± 0.1	46.4
Thr	79.8 ± 0.4	73.6 ± 0.3	82.0 ± 0.1	81.6 ± 0.3	48.3 ± 0.5	45.9 ± 0.1	42.5
Arg	80.9 ± 0.4	63.5 ± 0.3	76.8 ± 0.4	47.0 ± 0.5	48.5 ± 0.4	62.5 ± 0.3	41.9
Phe	73.0 ± 0.4	67.6 ± 0.3	53.0 ± 0.3	46.8 ± 0.3	45.9 ± 0.5	42.9 ± 0.3	41.2
Met	71.1 ± 0.1	65.8 ± 0.4	61.2 ± 0.1	57.2 ± 0.1	50.0 ± 0.3	42.4 ± 0.2	40.4
Try	73.0 ± 0.4	67.6 ± 0.4	59.9 ± 0.4	54.6 ± 0.3	54.4 ± 0.1	45.2 ± 0.3	38.1
Val	89.4 ± 0.2	85.2 ± 0.4	84.7 ± 0.4	84.7 ± 0.2	82.8 ± 0.4	56.6 ± 0.3	36.7
Ala	78.0 ± 0.4	56.8 ± 0.4	52.4 ± 0.2	67.4 ± 0.2	69.5 ± 0.3	72.8 ± 0.4	32.8
Pro	83.3 ± 0.2	70.2 ± 0.1	67.5 ± 0.3	60.3 ± 0.4	66.2 ± 0.5	67.4 ± 0.2	27.6
Gly	83.3 ± 0.2	94.7 ± 0.3	89.6 ± 0.5	77.8 ± 0.4	65.7 ± 0.4	62.6 ± 0.3	24.8
His	80.6 ± 0.3	71.2 ± 0.1	69.3 ± 0.2	63.7 ± 0.4	63.4 ± 0.2	63.6 ± 0.3	21.3
Ser	78.0 ± 0.4	82.9 ± 0.5	70.6 ± 0.4	66.0 ± 0.6	64.3 ± 0.2	64.5 ± 0.2	17.6
Asn	83.9 ± 0.5	82.6 ± 0.1	83.5 ± 0.4	84.4 ± 0.2	83.9 ± 0.3	85.2 ± 0.2	1.5
Lys	83.3 ± 0.2	96.2 ± 0.1	96.4 ± 0.2	96.5 ± 0.1	96.3 ± 0.3	89.5 ± 0.3	-7.4

and glutathione S-transferase [30]. Although selenium can induce the synthesis of metallothionein, the damage of metallothionein in scavenging free radicals could not be repaired due to very low level of intracellular glutathione content. Decrease in intracellular thiol will bring alteration of the lipid composition, which in turn may result in disrupting the organization of cell membrane, causing changes in fluidity, permeability and enzyme activities, inhibition of metabolic processes, and alterations of ion transport [31]. This may increase the leakage of glutathione from inner cells to outer broth. According to the results in Table 1, most glutathione was observed to be transferred to extracellular medium during the preparation of selenium-enriched yeast. Iizuka et al. had also found the similar phenomenon with *S. cerevisiae* [27].

It is well known that the addition of precursors of glutathione can improve the biosynthesis of this tripeptide. Other amino acids such as Met, Tyr and Asp, have positive effects on glutathione formation during selenium enrichment will result from their entering the main pathway after deamination or transamination. Hence the addition of these amino acids to the medium will consequently increase the metabolic flux to glycolysis and citric acid cycle, which, to some extent, would be in favor of excessive biosynthesis of ATP and precursors required for glutathione production. The increase of glutathione synthesis will be undoubtedly helpful to maintain a suitable oxidation-reduction environment together with the recovery of injured cell membrane caused by selenium assimilation. Some amino acids such

as Leu and Phe were also beneficial for the stabilization of cell membrane [32], which in turn would reduce the loss of intracellular glutathione.

Glutathione is an important antioxidant for protecting DNA, proteins, and other biomolecules such as metallothionein which has a stronger ability to remove hydroxyl radical than glutathione against oxidative damage by reactive oxygen species [16]. The redox system depended on glutathione had been shown to play an important role in the process against oxygen stress in *E. coli* and *S. cerevisiae*, and in maintaining the intracellular redox balance [15]. Adding certain amino acids appropriately as described above could increase intracellular glutathione content and improve distribution proportion of intracellular glutathione which will be helpful to maintain proper redox environment within cells.

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