Biosynthesis of Red Elemental Selenium Protein

Guojie Wu[1]，Xuegui Lin[1]，Yong Liu[2]，Jinwen Peng[1]，Jinhui Huang [3,a]

[1]School of chemistry and chemical engineering, Zhongkai University of Agriculture and Engineering,Guangzhou Guangdong 510225,China
[2]School of food and pharmaceutical engineering,Zhaoqing College,Zhaoqing Guangdong 526061,China
[3]School of materials science,Northwestern Polytechnical University,Xi'an Shanxi 710072,China

Abstract. The red elemental selenoprotein was biosynthesized by active dry yeast in this study. Through a single factor experiment and L9(34)-orthogonal test fermentation condition optimization, the selenium content was 15.72 mg/g under optimal condition. The optimum culture condition was as follows: the sodium selenite concentration (0.8 mg/mL), culture time (36 h), the yeast dosage (5 g), pH (5.5), culture temperature (30℃), and the medium Baume degree (8 °Bé). The analysis of amino acid species, content and morphology of the product showed that the red elemental selenoprotein was successfully synthesized.

1 Introduction

Selenium is one of the important trace elements in human body and plays an important role in the metabolism and immune function of the human body. In 1973, the World Health Organization (WHO) confirmed that selenium is an essential trace element for human and animal activities [1]. Selenium has good biological functions such as anti-oxidation, immunity, anti-cancer and anti-antagonism [2-4], and is called "a magical mineral with health effects". Selenium exists in the form of organic selenium and inorganic selenium in higher animals and plants, and organic selenium in animals and plants is combined with macromolecular proteins, amino acids, polysaccharides, etc. to form selenoproteins, selenoamino acids, selenopolysaccharides and the like. It accounts for more than 80% of total selenium, which is much higher than the amount of inorganic selenium.

In daily food consumption, the intake of selenium is very rare, unable to meet the normal needs of the body, often causing various diseases, so in order to meet the needs of the body, it is necessary to add exogenous selenium. At present, mainly tetravalent sodium selenite is used as an additive. Sodium selenite is not only highly toxic, but also causes damage to the body, and has low biological effects and low utilization rate of animal and plant, so it is easy to cause excessive addition. Cost increases and environmental pollution issues. A large number of studies have shown that zero-valent red elemental selenium has the advantages of low toxicity, high biological activity and high bioavailability, and is widely used in feed, health food, medicine and other fields [5]. However, red elemental selenium is easily agglomerated into gray or black elemental selenium, resulting in loss of biological activity. At present, most studies use direct reduction to reduce tetravalent sodium selenite to elemental selenium. This method is simple. Direct, but easy to cause agglomeration of selenium to denature, a small part can be stable, but the content is very low. Therefore, the development of a stable and reliable red elemental selenium product has great application prospects and significance, and the biological method has been applied more maturely in the fields of medicine and food, and is recognized as a safe and reliable method in the industry, and the obtained products for animals and plants has a high compatibility. Therefore, this study used a biological method to synthesize a stable, high content of red elemental selenium.

At present, the reducing bacteria of biochemical reduction of selenite are mainly: Escherichia coli, Ralstonia CH34, Rhodospirillum erythraea, capsular erythropolis, Pseudomonas fluorescens, Bacillus subtilis, etc. [6- 9]. However, the yeast reduced sodium selenite to produce red elemental selenoprotein is rarely reported. Therefore, we use yeast as a strain to study the synthesis of red elemental selenoprotein by yeast method, optimize the synthesis process, analyze the amino acid content and morphology of the product, and provide ideas for the biosynthesis of red elemental selenoprotein.

2 Materials and methods

2.1 Materials and Instruments

Yan brand hair active dry yeast was purchased from Wanjia Supermarket. Yeast extract powder and bacteriological protein were purchased from Guangdong Huankai Microbiology Technology Co., Ltd., and sodium selenite was purchased from Shandong West Asia Chemical Industry Co., Ltd., and its reagents were of analytical grade. AFS-2000 dual-channel atomic fluorescence spectrometer (Beijing Kechuang Haiguang

[a] Corresponding author: Jinhui Huang jinhui11818@outlook.com

Instrument Factory), XSP-5CA amino acid analyzer (Shanghai Optical Instrument Factory No. 6), EVO18 tungsten filament scanning electron microscope (SEM, Carl Zeiss, Germany), X-ray energy Spectrometer (EDS, EVO18, Germany), HSC.HA water bath thermostat (Jintan Guowang Experimental Instrument Factory).

2.2 Test methods

2.2.1 Biosynthesis of red elemental selenoprotein

Weigh 10 parts of 4g yeast, was added to 100mL wheat juice conical flask, 30 ℃, 150r / min activation culture for 48h; centrifuge to obtain yeast sludge, add to 100mL wort medium, add 10mg / mL selenite The sodium solution was 10 mL, fermented at 30 ℃, 150 r / min for 48 h, centrifuged at high speed, and dried at 80 ℃ for 12 h to obtain a red elemental selenoprotein product.

2.2.2 Determination of Selenium Content by Atomic Fluorescence Spectrometry

A sample of 0.1000 g was weighed, digested with nitric acid and perchloric acid, and diluted to 10,000 times with 50% hydrochloric acid. The test solution was measured using a two-channel atomic fluorescence spectrometer, and the data was recorded and the selenium content was calculated.

$$\text{Selenium content}(\text{mg/g}) = \frac{C \times V \times N}{M \times 10^6}$$

C is the concentration of selenium in the solution to be tested, mg/mL; V is the volume of the liquid to be tested, mL; N is the dilution multiple of the solution to be tested. M is the amount of yeast weighed, g.

2.2.3 Orthogonal test to optimize synthesis of red elemental selenoprotein

On the basis of single factor experiment, according to the orthogonal experimental design principle, the selenium content of the product was used as the optimization index, and the synthesis process of red elemental selenoprotein was optimized by selecting sodium selenite concentration, synthesis time, yeast dosage and pH4 factor.

2.2.4 Analysis of amino acid types, contents and morphology of products

The amino acid type and content of the product were analyzed by an amino acid analyzer; the surface morphology of the product was observed by scanning electron microscopy after the product was sprayed with gold.

2.3 Data Processing

Data were measured in parallel for three times, and SPSS20 was used for differential analysis. $P < 0.05$ was considered significant.

3 Results and discussion

3.1 Effect of sodium selenite concentration on selenium content.

It can be seen from Fig. 1 that as the concentration of sodium selenite increases, the selenium content of the product increases. When the concentration of sodium selenite is 0.9 mg/mL, the selenium content is the largest, and then decreases. The concentration of sodium selenite was significantly different for selenium content ($p<0.05$). Therefore, the concentration of sodium selenite was 0.9 mg/mL when synthesizing red elemental selenoprotein.

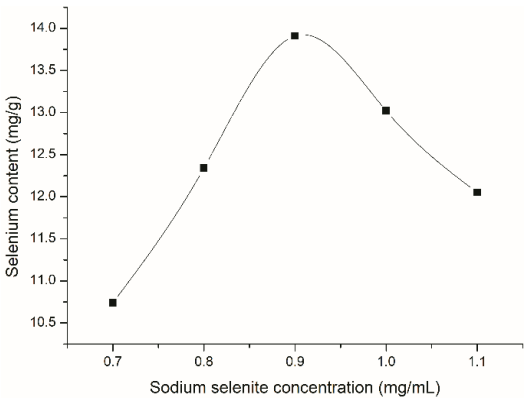


Fig.1 Effect of sodium selenite concentration on selenium content

3.2 Effect of yeast dosage on selenium content

It can be seen from Fig. 2 that as the amount of yeast increases, the selenium content of the product increases and reaches a maximum at 4 g, and then shows a downward trend. The amount of yeast used was significantly different for selenium content ($p<0.05$). Therefore, it is appropriate to use 4g of yeast when synthesizing red elemental selenoprotein.

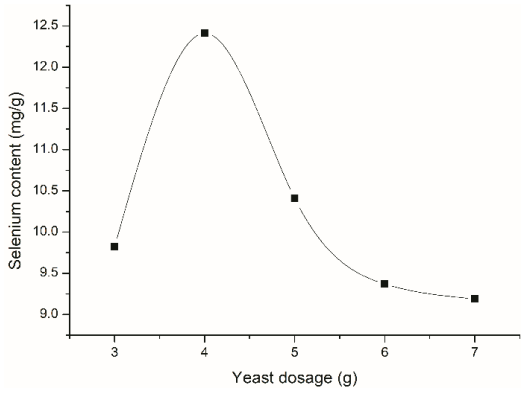


Fig.2 Effect of yeast dosage on selenium content

3.3 Effect of synthesis time on selenium content

It can be seen from Fig. 3 that the selenium content of the product increases first and then decreases with the increase of the synthesis time, and reaches a peak at 48 hours. The synthesis time level was significantly

different for selenium content ($p<0.05$). Therefore, the synthesis time was 48h when synthesizing red elemental selenoprotein.

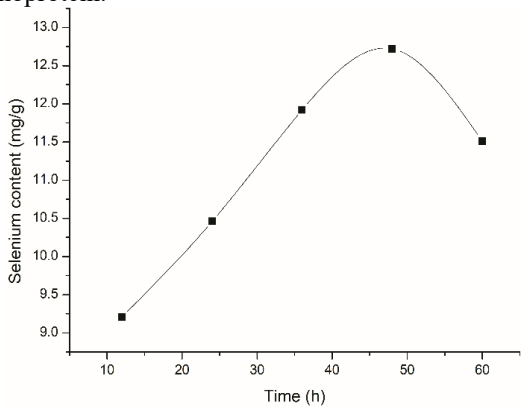


Fig.3 Effect of synthesis time on selenium content

3.4 Effect of pH on selenium content

It can be seen from Fig. 4 that the selenium content of the product first increases and then decreases with the increase of the pH of the fermentation liquid, and reaches a peak at pH 5.5. The pH level was significantly different for selenium content ($p<0.05$). Therefore, a pH of 5.5 was suitable when synthesizing red elemental selenoprotein.

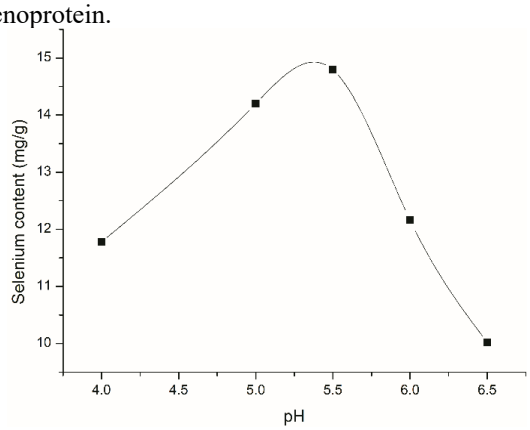


Fig.4 Effect of pH on selenium content

3.5 Effect of synthesis temperature on selenium content

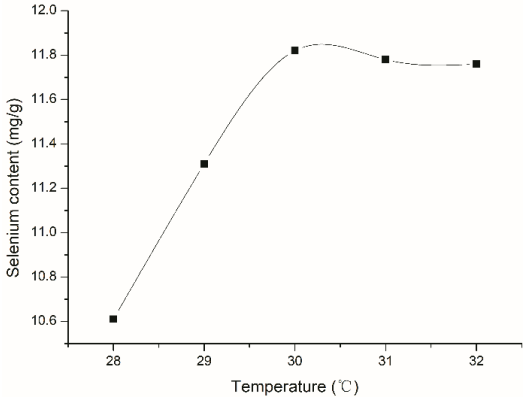


Fig.5 Effect of synthesis temperature on selenium content

It can be seen from Fig. 5 that with the increase of synthesis temperature, the selenium content of the product gradually increases, and it is basically stable at 30 ℃. After that, the synthesis temperature is not significantly different from the selenium content ($p > 0.05$). Therefore, the synthesis of red elemental selenoprotein was fixed in a temperature of 30 ℃.

3.6 Effect of medium Baume degree on selenium content

It can be seen from Fig. 6 that with the increase of the medium Baume degree（°Bé）, the selenium content of the product gradually increases, and it is basically stable at 8 °Bé. After that, the s medium Baume degree is not significantly different from the selenium content ($p>0.05$). Therefore, the synthesis of red elemental selenoprotein was fixed at a medium Baume degree of 8 °Bé for optimization experiments.

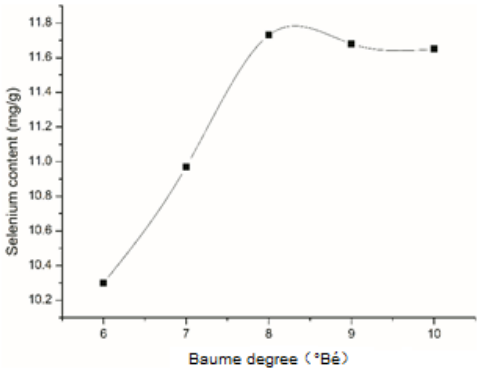


Fig.6 Effect of medium Baume degree on selenium content

3.7 Orthogonal test optimization of synthetic red elemental selenoprotein

Based on the single factor test, sodium selenite concentration (A, mg/mL), synthesis time (B, h), yeast dosage (C, g) and pH (D) were used independent variables, and the selenium content (Y, mg/g) was used as the optimization index. The sodium selenite concentration, synthesis time, yeast dosage and pH are optimized by 4 factors and 3 levels orthogonal test to synthesize the red elemental selenoprotein, and the test results and visual analysis are shown in Table 1.

Table 1. Design and visual analysis of $L_9(3^4)$ orthogonal test

Number	*A*	*B*	*C*	*D*	*Y*
1	1(0.8)	1(36)	1(3)	1(5.0)	12.27
2	1	2(48)	2(4)	2(5.5)	15.39
3	1	3(60)	3(5)	3(6.0)	15.22
4	2(0.9)	1	2	3	15.41
5	2	2	3	1	12.83
6	2	3	1	2	11.85
7	3(1.0)	1	3	2	15.65
8	3	2	1	3	10.83
9	3	3	2	1	10.51
k_1	14.293	14.443	11.650	11.870	

k_2	13.363	13.017	13.770	14.297
k_3	12.330	12.527	14.567	13.820
R	1.963	1.916	2.917	2.427

The magnitude of the range R value indicates the magnitude of the effect of changes in the range of the test on the test results. The larger the R value, the more significant the influence of the listed factors on the test results. It can be seen from Table 1 that the order of influence of four factors on selenium content is yeast dosage (C)> pH(D)> sodium selenite concentration(A) > synthesis time(B), and the optimal synthesis process of red elemental selenoprotein is $A_1B_1C_3D_2$. Three parallel tests were carried out according to the optimal synthesis process, and the average value of the selenium content of the product was 15.72 mg/g.

3.8 Analysis of amino acid types and contents of products

The amino acid type and content of the product and yeast were determined by an amino acid analyzer as shown in Table 2. As can be seen from Table 2, the amino acid content of the product is slightly higher than that of the yeast, and the total amount of other amino acids including the amino acid is significantly lower than that of the yeast; in addition, selenium is not detected in the yeast, but the selenium content in the product is 1.57 %. It shows that the red elemental selenoprotein was successfully synthesized.

Tab.2. Types and contents of amino acids in products and yeast

Types	Yeast（%）[10]	Products（%）
Phe	2.04	1.84
Ala	2.46	2.12
Met	0.77	0.78
Pro	1.75	1.35
Gly	1.98	1.61
Glu	6.32	3.61
Cys	0.31	0.22
Arg	2.20	1.70
Lys	3.58	3.14
Tyr	1.44	1.30
Leu	3.10	2.81
Ser	2.10	1.79
Thr	2.24	1.99
Asp	4.60	3.70
Val	2.26	1.97
Ile	2.04	1.88
His	1.03	1.01
Total amino acid	40.22	32.82
Total crude protein	40.40	40.48
Se	Not detected	1.57

3.9 Product morphology analysis

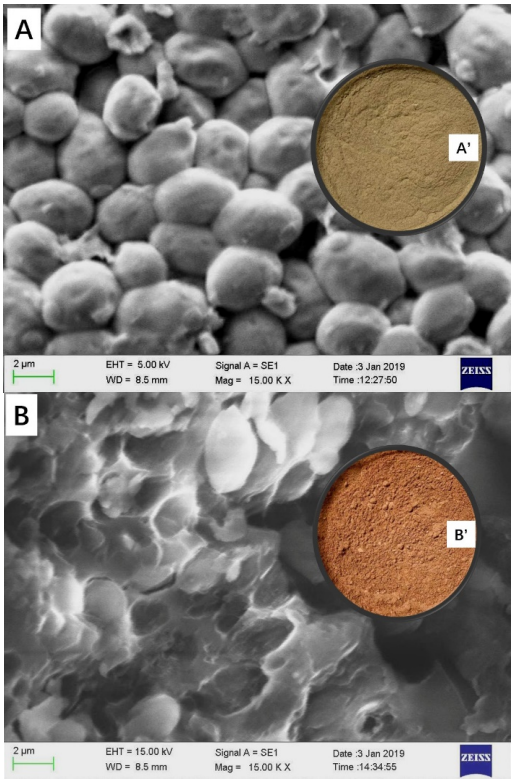
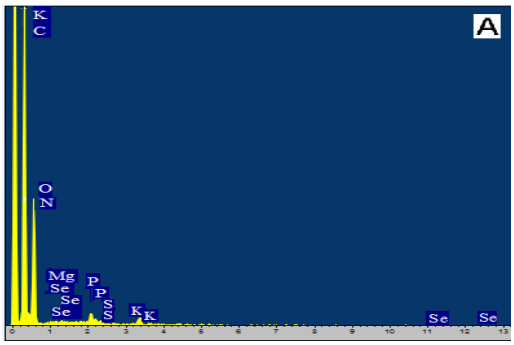


Fig.7 Optical photographs(A',B') and SEM images(A,B) of yeast(A) and red selenoprotein(B)

Figure 7 is an optical photograph and an electron micrograph of yeast and product. It can be observed from the figure that the yeast is light yellow and the red selenoprotein product is red. From the electron micrograph of Fig. 7, a typical spheroidal yeast with a uniform yeast morphology, uniform size and smooth surface can be observed; while partially intact yeast can still be seen in the product, most of the yeast has been broken due to yeast, caused by biological reaction with sodium selenite.



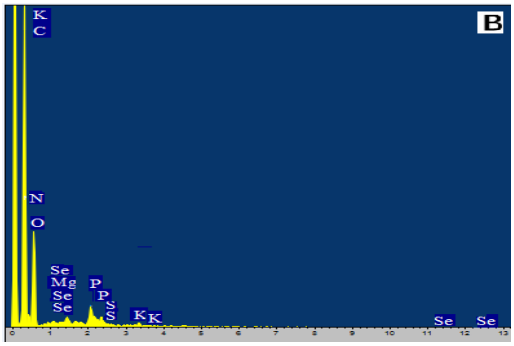


Fig.8 EDS spectrum of yeast(A) and red selenoproteinb(B)

Surface element analysis of yeast and products was carried out. From the EDS spectrum of Fig. 8, it can be seen that there is no characteristic peak of selenium in yeast, and a distinct selenium characteristic peak appears on the surface of the product. It indicated that selenium yeast successfully retained selenium in the cells, but it can be seen from the results of component analysis in Table 3 that the selenium content is significantly lower than the measured results, which may be due to the fact that the yeast biotransformed selenoprotein is mainly in yeast cells. The EDS elemental analysis is only for the surface of the sample, only the selenoprotein exposed by the broken yeast cells is detected, and the selenoprotein in the body can be detected. From the electron micrograph analysis of Fig. 7, it is known that there are many unbroken yeasts in the product, and therefore, the selenium content is lower than the measured content.

Tab.3 Element contents of EDS spectrum analysis

Content elements	Weight（%）	
	Yeast[10]	Red elenoprotein
C K	50.51	53.24
N K	8.44	12.30
O K	38.97	31.60
Mg K	0.09	0.08
P K	0.76	1.12
S K	0.19	0.61
K K	1.03	0.36
Se K	0.02	0.68
Total	100	100

4 Conclusions

Based on the analysis of single factor experiments, the orthogonal synthesis was used to optimize the synthesis process of red elemental selenoprotein. The order of influence of various factors on selenium content was yeast dosage > pH > sodium selenite concentration > synthesis time. The optimal synthesis process of red elemental selenoprotein is sodium selenite concentration of 0.8 mg/mL, synthesis time of 36 h, yeast dosage of 5 g, pH of 5.5, synthesis temperature of 30 ℃, medium Baume degree of 8 °Bé . The selenium content of the product under this condition is 15.72 mg/g. The amino acid species, content and morphology of the product showed that the red elemental selenoprotein was successfully synthesized.

Funding:

This work was supported by the Guangdong Science and Technology Planning Project (No. 2016A010105023) and the Guangzhou Science and Technology Planning Project (No. 201704020055).

References

1. WHO Tech. Trace elements in human nutrition. (1973)
2. Miller S, Walker S W, Arthur J R, et al. Selenite protects human endothelial cells from oxidative damage and induces thioredoxin reductase. Clinical Science, 100, 5 (2001)
3. Turło J, Gutkowska B, Herold F. Effect of selenium enrichment on antioxidant activities and chemical composition of Lentinula edodes (Berk.) Pegl. mycelial extracts. Food and Chemical Toxicology, 48, 4 (2010)
4. Malinowska E, Krzyczkowski W, Herold F, et al. Biosynthesis of selenium-containing Polysaccharides with antioxidant activity in liquid culture of Hericium erinaceum. Enzyme and Microbial Technology, 44, 5 (2009)
5. Dongliang W, Wei Q, Bo H,et al.Screening and identification of photosynthetic bacterial strains of red simple selenium produced by reducing selenite. Journal of Microbiology, 47, 1 (2007)
6. Roux M, Sarret G, Pignot-Paintrand I, et al. Mobilization of Selenite by Ralstonia metallidurans CH34. Applied and Environmental Microbiology, 67, 2 (2001)
7. Turner R J, Weiner J H, Taylor D E. Selenium metabolism in Escherichia coli. Biometals, 11, 3 (1998)
8. Kessi J. Enzymic systems proposed to be involved in the dissimilatory reduction of selenite in the purple non-sulfur bacteria Rhodospirillum rubrum and Rhodobacter capsulatus. Microbiology, 152 (2006)
9. Garbisu C, Ishii T, Leighton T, et al. Bacterial reduction of selenite to elemental selenium. Chemical Geology, 132 (1996)
10. Guojie W, Fei L, Xiaowen S,et al.Preparation of Selenium-enriched Yeast by re-using Discarded Saccharomyces cerevisiae from the Beer Industry for Se-Supplemented Fodder Applications .applied sciences, 3777, 9 (2019)