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Culture Optimization and Amino Acid Composition of Cr-Enriched Mycelia of *Pleurotus cornucopiae* SD-01

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Summary

Chromium(III) is an essential trace element for humans and animals. *Pleutorus cornucopiae* SD-01 is a nutritional and functional mushroom containing many kinds of bioactive ingredients. The aims of this work are to optimize the conditions of *P. cornucopiae* SD-01 cultivation with Cr enrichment in submerged culture by determining the dry cell mass, Cr content in mycelia and the rate of Cr enrichment, and to analyze the amino acid composition of Cr-enriched mycelia. The optimal medium contained (in g/L): potato 200, sucrose 25, yeast extract 4, KH_2PO_4 1 and $MgSO_4 \cdot 7H_2O$ 1. The optimum parameters of liquid culture were temperature 25 °C, cultivation time 6 days, the volume of the medium 100 mL, rotation speed 160 rpm and initial pH=6.5. Under the optimized conditions, the values of the dry cell mass, Cr content in mycelia and the rate of Cr enrichment were (6.63±0.35) g/L, (3670±211) μg/g and (12.15±1.01) % respectively, which were by (23.23±1.22), (18.19±1.06) and (45.68±2.67) % respectively, higher than those in the control. Chromium(III) in Cr-enriched mycelia was mainly combined with protein and polysaccharide. The contents of total amino acids and essential amino acids in Cr-enriched mycelia were increased by (31.25±0.58) and (44.26±0.76) %, respectively.

Key words: chromium(III), Cr-enrichment, *Pleutorus cornucopiae* SD-01, culture optimization, amino acid composition

Introduction

Trivalent chromium (Cr(III)) is generally believed to be an essential trace element and widely used as a nutritional supplement for humans and animals (*1*). As a bioactive ingredient of glucose tolerance factor (GTF), it has been shown to increase insulin sensitivity and lower glycated hemoglobin levels, making it attractive as a potential therapeutic treatment for gestational diabetes (*2*). It also influences the metabolism of carbohydrates (*3–5*), lipids (*6*), proteins (*1,7,8*), nucleic acids (*9,10*) and the activity of glycogen synthetase (*11*) by cooperating with GFT.

Many mushrooms can efficiently absorb and transform trace elements in media from inorganic into organic forms, which are more easily utilized by human body (*12*). With respect to the Cr-enriched mushrooms, there have been very few reports until now (*13,14*). *Pleurotus cornucopiae* Paul. ex Pers. is one of the cultivated mushrooms recommended in developing countries by Food and Agriculture Organization (FAO), containing a lot of trace elements, protein, essential amino acids, dietary fibre, vitamins and polysaccharides. It has been reported that *P. cornucopiae* had preventive and remedial effect on some diseases, such as coronary heart diseases, hyperglycemia, solid tumor S 180, ascites caused by cancer, *etc.* (*15,16*). The Cr-enriched *P. cornucopiae* should simultaneously have the nutritious and physiological functions of *P. cornucopiae* and Cr, and make it possible to supply sufficient Cr to the organism effectively.

However, artificial solid-state cultivation of Cr-enriched fruiting body not only needs a long period of production, but also obtains a low percentage of Cr ac-

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cumulation (*13*). Information is lacking with regard to Cr-enriched mycelia of *P. cornucopiae* in submerged culture.

The objectives of this study are to optimize the medium composition and the cultivation conditions of Cr enrichment by *P. cornucopiae* SD-01 in submerged culture with the dry cell mass, Cr content in mycelia and the rate of Cr enrichment as the main indices on the basis of the orthogonal experimental design and single-factor tests, and to investigate the Cr distribution and amino acid composition of Cr-enriched mycelia.

Materials and Methods

Microorganism

A strain of *Pleurotus cornucopiae* SD-01 was provided by our laboratory and used in this experiment. It was incubated on synthetic potato dextrose agar (PDA) plates for 10 days at 25 °C, maintained at 4 °C and subcultured every 3 months.

Determination of Cr concentration in liquid media

All experiments of liquid cultivation were carried out in 250-mL Erlenmeyer flasks. To determine the optimal Cr concentration in the liquid medium, the Cr ions were separately added to the basal medium and their final concentrations were (in μg/mL): 20, 40, 60, 80, 100, 200, 300, 500, 800 and 1000, taking no Cr addition to the medium as a comparison (0 μg/mL). The source of Cr was $CrCl_3 \cdot 6H_2O$. The composition of the basal medium was (in g/L): potato 150, sucrose 20, peptone 2, KH_2PO_4 1 and $MgSO_4 \cdot 7H_2O$ 0.5 with natural pH. Every flask containing 100 mL of the basal medium was inoculated with a 0.5-cm^2 mycelial block of *P. cornucopiae* SD-01 from the above maintained plates and incubated on a rotary shaker (Anting Biotech, Shanghai, PR China) at 160 rpm and 25 °C for 6 days.

Medium optimization for Cr enrichment

Potato was used as a basic substrate in the medium nutrient composition. The additional supplements of carbon (glucose, sucrose, maltose, lactose, fructose or soluble starch) and nitrogen sources (peptone, yeast extract, beef extract, NH_4NO_3, $(NH_4)_2SO_4$, $NaNO_3$, NH_4Cl or urea) were first selected and then screened with single-factor experiments. A subsequent five-factor-three-level orthogonal test was applied to optimize the medium composition for Cr enrichment after finding the optimal carbon and nitrogen sources, while the cultivation conditions were the same as mentioned above.

All chemicals used in this study were of analytical reagent grade, purchased from local chemical suppliers in China.

Optimization of cultivation conditions for Cr enrichment

Cultivation time (3, 4, 5, 6, 7, 8 and 9 days), temperature (20, 25, 30 and 35 °C), initial pH (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0), rotation speed (100, 120, 140, 160, 180 and 200 rpm) and volume of the medium (50, 100 and 150 mL) in 250-mL flasks were investigated in this experiment by single-factor tests for Cr enrichment optimization. All trials were carried out in the optimum liquid medium obtained above.

Measurement of dry cell mass, Cr content in mycelia and the rate of Cr enrichment

The original mycelial block of inoculum in liquid medium was discarded and the Cr-enriched mycelia of *P. cornucopiae* SD-01 were obtained by centrifugation (3000×*g*, 20 min) in a centrifuge (Beckman Instruments, Palo Alto, CA, USA) at the end of cultivation time according to the experimental design. Dry cell mass of Cr-enriched mycelia was measured after rinsing the mycelium precipitate with distilled water for three times and then drying to a constant mass at 60 °C in an oven for about 48 h.

In order to thoroughly remove the unabsorbed Cr and evaluate the Cr content in the mycelia, Cr-enriched mycelium powder (0.5 g) was dialyzed repeatedly in double distilled water containing 0.02 % (by volume) sodium azide until there was no amino acid found using ninhydrin reaction. The concentrated solution obtained from the dialysis bag (Putian Biotech, Beijing, PR China) was agitated repeatedly. After centrifugation (3000×*g*, 20 min), the sediment was mixed with 8 mL of perchloric acid (12.5 M) and nitric acid (16 M) mixture (8:1, by volume) and digested completely at 140 °C for 1 h until the solution turned colourless. The cooled liquid was diluted to a constant volume of 100 mL with 1 M HNO_3. The content of Cr in the Cr-enriched mycelia of *P. cornucopiae* SD-01 was measured by atomic absorption spectrometry (AAS; PerkinElmer Analyst 800 model atomic absorption spectrometer, PerkinElmer, Waltham, MA, USA). The rate of Cr enrichment in the mycelia was calculated by the following formula:

$$w(\text{Cr accumulated in mycelia})/(\mu g/g) - w(\text{Cr in control})/(\mu g/g) \times (c(\text{Cr-enriched mycelia})/g/c(\text{Cr in media})/\mu g) \times 100 \quad /1/$$

where c is concentration in mol/L.

Determination of organic Cr and the content of Cr in the main components of Cr-enriched mycelia

Cr-enriched mycelium powder (0.5 g) was dialyzed repeatedly with double distilled water containing 0.02 % (by volume) of sodium azide until there was no amino acid found using ninhydrin reaction. Then, the content of Cr was determined by atomic absorption spectrometry (AAS).

Mycelium powder (2 g) was dissolved in 50 mL of NaCl (2 M) and extracted at 100 °C (*17*). Protein was removed from the prepared supernatant by the method of Yang and Zhou (*18*). Before adjusting the pH to 2.0, the liquid was cooled in ice water for 30 min. After adding 95 % ethanol, the precipitate of nucleic acids was obtained by centrifugation at 3000×*g* for 15 min. The content of Cr in nucleic acid was determined by AAS.

The residue from the previous step was distilled with 50 mL of water for 2 h. After filtration, quadruple volume of 95 % ethanol was added into the supernatant

and the mixture was stored for 1 day at 4 °C in the dark. The polysaccharide precipitate was obtained at 3000×*g* for 15 min.

Mycelium powder (2 g) was put into 100 mL of NaOH (0.25 M). After distillation at 50 °C for 4 h, $(NH_4)_2SO_4$ was added until the saturated degree was 95 %. After adding the mixture of ethanol and ether (2:1, by volume), the protein sediment was obtained by centrifugation at 3000×*g* for 15 min. The content of Cr in the protein was measured by AAS.

Determination of amino acids in Cr-enriched mycelia

Fungal powder (100 mg) was put into a hydrolyzation tube with HCl (6 M) for 1 day at 110 °C and subsequently diluted with distilled water to a constant volume of 100 mL. The liquid (2 mL) from the previous step was vacuum dried under low pressure at 60 °C. After adding 2 mL of 0.02 M HCl, the content of amino acids (50-μL sample) was determined by an automatic amino acid analyzer (Hitachi L-8900, Japan).

The liquid sample was adjusted to pH=7 and fixed to a volume of 100 mL with 4 M urea (pH=11) after being hydrolyzed with NaOH (5 M) for 20 h at 110 °C. Tryptophan (Trp) was determined by the method of Landry *et al*. (*19*).

Statistical analysis

All experiments were carried out in triplicate and all the data were expressed as means±S.D. (standard deviation). The statistical significance was evaluated using Student's *t*-test and $p<0.05$ was taken as significant.

Results and Discussion

Determination of Cr concentration added to the basal medium

The results of the optimization of Cr concentration added to the basal medium are shown in Table 1, while no detectable Cr contents were observed in the basal medium before the addition of Cr. The values of dry cell mass and the rate of Cr enrichment increased gradually along with the increasing Cr concentration (0–200 μg/mL) in the media and declined rapidly when the concentration of Cr exceeded 300 μg/mL, indicating that high concentration of Cr could evidently inhibit the mycelium growth and Cr accumulation. At the concentration of Cr of 200 μg/mL, the maximum production of dry cell mass and the rate of Cr enrichment reached (5.38±0.36) g/L and (8.34±0.72) %, respectively, while the Cr content in the mycelium was (3100±203) μg/g. The value of optimal Cr concentration (200 μg/mL) added to the liquid medium was in accordance with that reported in literature (*13*,*14*).

Optimization of the medium and cultivation conditions for Cr enrichment

The effects of various carbon and nitrogen sources on the dry cell mass, Cr content in mycelia and the rate of Cr enrichment are described in Table 2. Some of the carbon and nitrogen sources had a positive effect on Cr enrichment in submerged culture by *P. cornucopiae* SD-01. The peak values of the three parameters were (4.05±0.26) g/L, (3290±287) μg/g and (6.65±0.41) %, respectively, obtained in the presence of sucrose in the medium. The analysis of variance (ANOVA) showed the different effects of the six carbon sources on dry cell mass ($p<0.05$), Cr content in the mycelia ($p<0.01$) and the rate of Cr enrichment ($p<0.05$). When yeast extract was used as nitrogen source, the highest values of dry cell mass, Cr content in mycelia and the rate of Cr enrichment were

Table 1. Effect of Cr on dry cell mass, Cr content in mycelia and rate of Cr enrichment of *P. cornucopiae* SD-01

γ(Cr)	*γ*(dry cell mass)	*w*(Cr content in mycelia)	*w*(rate of Cr enrichment)
μg/mL	g/L	μg/g	%
0	3.25±0.26	nd	0.00
20	3.38±0.31	130±11	2.14±0.17
40	3.71±0.43	370±26	3.42±0.24
60	4.08±0.39	680±50	4.65±0.36
80	4.16±0.33	1310±99	6.81±0.49
100	4.69±0.38	1630±113	7.65±0.61
200	5.38±0.36	3100±203	8.34±0.72
300	4.07±0.29	3600±263	4.89±0.29
500	3.65±0.21	3560±316	2.60±0.21
800	1.06±0.18	2140±232	0.28±0.05
1000	0.12±0.05	150±16	<0.01

nd – not detectable

Table 2. Effect of carbon and nitrogen sources on dry cell mass, Cr content in mycelia and the rate of Cr enrichment of *P. cornucopiae* SD-01

Source	*γ*(dry cell mass)	*w*(Cr content in mycelia)	*w*(rate of Cr enrichment)
	g/L	μg/g	%
Carbon sources	*	**	*
Glucose	3.59±0.23	2980±226	5.35±0.39
Sucrose	4.05±0.26	3290±287	6.65±0.41
Maltose	3.82±0.27	2690±254	5.20±0.44
Lactose	2.93±0.33	2320±220	3.39±0.37
Fructose	1.75±0.25	1460±147	1.27±0.18
Soluble starch	2.57±0.34	2150±210	2.76±0.33
Nitrogen sources	*	**	*
Peptone	3.64±0.41	2820±236	5.12±0.45
Yeast extract	3.86±0.33	3420±286	6.99±0.48
Beef extract	3.69±0.39	2990±279	5.51±0.51
NH_4NO_3	2.93±0.32	1880±197	2.75±0.24
$(NH_4)_2SO_4$	3.17±0.29	2480±231	3.93±0.27
$NaNO_3$	1.16±0.27	690±56	0.40±0.03
NH_4Cl	2.89±0.34	1630±154	2.36±0.19
Urea	0.98±0.16	460±38	0.23±0.02

*$p<0.05$, **$p<0.01$

(3.86±0.33) g/L, (3420±286) μg/g and (6.99±0.48) %, respectively. ANOVA showed that the results of significance for eight nitrogen sources were similar to those for carbon sources.

Based on the previous results, sucrose and yeast extract used as optimal carbon and nitrogen sources were selected and applied to optimize the medium composition in orthogonal experiments. The design of five-factor-three-level orthogonal tests is shown in Table 3 and the results are described in Table 4.

Table 3. Five-factor-three-level design of the orthogonal experiment

Levels	γ/(g/L)				
	A	B	C	D	E
	Potato	Sucrose	Yeast extract	KH_2PO_4	$MgSO_4 \cdot 7H_2O$
1	100	15	2	1	0.5
2	150	20	4	2	1.0
3	200	25	6	3	1.5

Among these substrates, potato, sucrose and yeast extract showed significant influence on Cr content in mycelia ($p<0.01$) and the rate of Cr enrichment ($p<0.01$). The composition of the Cr-enriched medium was obtained as follows (in g/L): potato 200, sucrose 25, yeast extract 4, KH_2PO_4 1, $MgSO_4 \cdot 7H_2O$ 1. Under these conditions, the dry cell mass, Cr content in mycelia and the rate of Cr enrichment reached (5.69±0.29) g/L, (3570±286) μg/g and (10.13±0.59) %, respectively, which is (5.76±0.16), (14.95±0.21) and (21.46±0.87) % higher than those in the basal medium, respectively.

Table 5 shows that *P. cornucopiae* SD-01 could grow at initial pH value ranging from 4.5 to 9, and the dry cell mass ((5.37±0.24) g/L, $p<0.05$), Cr content in mycelia ((2740±204) μg/g, $p<0.05$) and the rate of Cr enrichment ((7.35±0.33) %, $p<0.05$) reached their optimum values at initial pH=6.5. Similar results were reported by Liu *et al.* (*14*). Although the dry cell mass was lower at 25 °C ((4.86±0.26) g/L, $p<0.05$) than at 30 °C ((5.41±0.33) g/L, $p<0.05$), the Cr content in mycelia ((2970±266) μg/g, $p<0.05$) and the rate of Cr enrichment ((7.22±0.31) %, $p<0.05$) were obviously higher.

Table 4. Results of the orthogonal experiments for medium optimization

No.	A	B	C	D	E	γ(dry cell mass)	w(Cr content in mycelia)	w(rate of Cr enrichment)
						g/L	μg/g	%
1	1	1	1	1	1	2.86±0.23	1540±134	2.19±0.19
2	1	1	1	1	2	2.93±0.21	1620±138	2.36±0.16
3	1	1	1	1	3	2.98±0.19	1640±130	2.44±0.21
4	1	2	2	2	1	3.12±0.24	2440±188	3.81±0.18
5	1	2	2	2	2	3.16±0.26	3010±265	4.76±0.23
6	1	2	2	2	3	5.12±0.30	2870±258	7.35±0.37
7	1	3	3	3	1	3.28±0.28	2890±266	4.74±0.29
8	1	3	3	3	2	4.94±0.35	2420±223	5.97±0.34
9	1	3	3	3	3	3.08±0.22	2880±257	4.43±0.28
10	2	1	2	3	1	4.07±0.28	1640±140	3.85±0.21
11	2	1	2	3	2	3.81±0.17	2000±168	3.81±0.25
12	2	1	2	3	3	3.45±0.28	1840±158	3.17±0.22
13	2	2	3	1	1	3.18±0.26	3020±274	4.81±0.24
14	2	2	3	1	2	3.45±0.23	2990±269	5.15±0.27
15	2	2	3	1	3	3.92±0.31	2870±263	5.62±0.33
16	2	3	1	2	1	5.13±0.33	1850±154	3.86±0.21
17	2	3	1	2	2	5.32±0.35	1460±123	3.87±0.27
18	2	3	1	2	3	4.55±0.29	2020±176	4.58±0.32
19	3	1	3	2	1	4.18±0.32	2680±230	2.69±0.22
20	3	1	3	2	2	3.52±0.27	3460±296	6.43±0.34
21	3	1	3	2	3	4.25±0.29	2940±278	6.24±0.45
22	3	2	1	3	1	3.65±0.19	2930±255	5.34±0.29
23	3	2	1	3	2	4.81±0.37	3020±259	7.26±0.38
24	3	2	1	3	3	3.05±0.28	3210±286	4.89±0.26
25	3	3	2	1	1	5.36±0.36	3190±279	8.53±0.47
26	3	3	2	1	2	5.69±0.29	3570±286	10.13±0.59
27	3	3	2	1	3	4.73±0.34	3220±267	7.61±0.32

Table 4. – continued

No.	A	B	C	D	E	γ(dry cell mass) g/L	w(Cr content in mycelia) μg/g	w(rate of Cr enrichment) %
K_1	3.51	3.56	3.92	3.83	3.87			
K_2	4.10	3.72	4.21	4.26	4.11			
K_3	4.29	4.61	3.76	3.79	3.90		Σ=100.76	
R	0.79	1.05	0.45	0.47	0.24			
$p>F^a$	0.0607	0.0169*	0.2978	0.4655	0.5051			
K_1'	2367.36	2171.06	2141.63	2614.95	2463.45			
K_2'	2186.24	2928.32	2627.91	2546.59	2624.39			
K_3'	3142.49	2596.73	2926.57	2534.57	2608.27		Σ=69264.95	
R′	956.25	757.26	784.94	80.38	160.94			
$p>F^b$	<0.0001**	0.0002**	0.0001**	0.8421	0.5092			
K_1''	4.23	3.69	4.09	5.39	4.42			
K_2''	4.30	5.44	5.86	4.84	5.49			
K_3''	6.56	5.94	5.12	4.83	5.15		Σ=135.59	
R″	2.31	2.25	1.77	0.56	1.07			
$p>F^c$	0.0004**	0.0011**	0.0093**	0.3751	0.1072			

*$p<0.05$, **$p<0.01$

Along with the prolongation of cultivation time, the dry cell mass and the rate of Cr enrichment increased remarkably from 3 to 6 days ($p<0.01$) and the maximal Cr content in mycelia ((2910±277) μg/g) occurred on day 8 ($p<0.01$). To shorten the fermentation time and obtain higher dry cell mass, Cr content in mycelia and the rate of Cr enrichment, day 6 was chosen as the optimal cultivation time. It can be seen from Table 5 that the optimal values of the medium volume and rotation speed were: 100 mL and 160 rpm, respectively. As a result, the optimal cultivation conditions for Cr-enrichment by *P. cornucopiae* SD-01 were: temperature 25 °C, cultivation time 6 days, initial pH=6.5, volume of medium 100 mL and rotation speed 160 rpm.

Table 5. Effect of cultivation conditions on the dry cell mass, Cr content in mycelia and the rate of Cr enrichment of *P. cornucopiae* SD-01

Cultivation condition	γ(dry cell mass) g/L	w(Cr content in mycelia) μg/g	w(rate of Cr enrichment) %
Cultivation time/day	**	**	**
3	1.49±0.15	1360±115	1.01±0.08
4	2.57±0.19	1870±156	2.40±0.12
5	3.65±0.21	2160±177	3.95±0.22
6	5.28±0.28	2760±226	7.28±0.35
7	4.08±0.22	2900±266	5.91±0.46
8	2.69±0.16	2910±277	3.91±0.28
9	2.18±0.11	2010±187	2.19±0.09
Temperature/°C	*	*	*
20	3.12±0.27	1810±159	2.83±0.14
25	4.86±0.26	2970±266	7.22±0.31
30	5.41±0.33	2120±185	5.75±0.33
35	4.32±0.24	1430±123	3.08±0.27
Initial pH	*	*	*
4.5	3.04±0.25	1430±127	2.17±0.19
5.0	3.16±0.28	1540±132	2.43±0.17
5.5	3.68±0.23	1900±168	3.49±0.22
6.0	4.46±0.22	2220±189	4.94±0.29
6.5	5.37±0.24	2740±204	7.35±0.33
Initial pH	*	*	*
7.0	5.32±0.31	2520±226	6.69±0.36
7.5	4.23±0.25	1660±147	3.51±0.27
8.0	3.06±0.22	1100±896	1.69±0.11
8.5	1.01±0.09	1090±866	0.55±0.06
9.0	0.65±0.03	990±664	0.32±0.03
Volume of medium/mL	*	*	**
50	2.54±0.17	2470±209	3.13±0.22
100	4.89±0.28	2970±247	7.27±0.34
150	3.12±0.22	2990±165	3.10±0.25
Rotation speed/rpm	**	**	**
100	2.18±0.17	1580±140	1.72±0.11
120	3.26±0.21	1890±166	3.09±0.25
140	4.12±0.28	2250±185	4.63±0.21
160	5.48±0.32	3130±191	8.56±0.36
180	5.08±0.29	3040±247	7.71±0.52
200	4.18±0.26	2830±155	5.91±0.39

*$p<0.05$, **$p<0.01$

Validation of the model

Under the optimal medium composition and cultivation conditions, the values of dry cell mass, Cr content in mycelia and the rate of Cr enrichment were (6.63±0.35) g/L, (3670±211) μg/g and (12.15±1.01) %, respectively, which were (23.23±1.22), (18.19±1.06) and (45.68±2.67) % higher than the control, respectively.

Cr distribution and amino acids in Cr-enriched mycelia

The mass fraction of organic Cr in *P. cornucopiae* SD-01 was (92.46±0.82) %. It can be seen from Table 6 that chromium in Cr-enriched mycelia was mainly combined with protein and polysaccharide, which can be used as the main supplemental chromium sources for humans and animals. The content of Cr in protein and polysaccharide relative to organic Cr in the mycelia was (67.25±1.16) and (15.73±0.85) %, respectively.

Table 7 shows that the contents of total amino acids and essential amino acids in Cr-enriched mycelia of *P. cornucopiae* SD-01 compared to the control were increased by (31.25±0.58) and (44.26±0.76) %, respectively, indicating that the optimal Cr concentrations contributed to promoting protein and amino acid biosynthesis.

Table 6. Cr content in the main components of Cr-enriched mycelia

Component of mycelia	*w*(Cr)/(μg/g)	*w*(Cr/organic Cr)/%
Proteins	2280±18	67.25±1.16
Polysaccharides	530±6	15.73±0.85
Nucleic acids	70±2	2.17±0.04
Other	500±5	14.85±0.13

Table 7. Content of amino acids in Cr-enriched mycelia of *P. cornucopiae* SD-01

Amino acid	*w*(control)	*w*(Cr-enriched mycelia)
	mg/g	mg/g
Alanine	5.6±0.2	6.1±0.2
Arginine	1.7±0.1	1.8±0.1
Aspartic acid	9.5±0.3	9.8±0.2
Cysteine	4.9±0.2	2.8±0.2
Glutamic acid	12.9±0.8	19.7±0.8
Glycin	10.2±0.2	13.8±0.3
Histidine	6.4±0.1	5.7±0.1
Isoleucine[a]	14.7±0.9	25.6±0.8
Leucine[a]	16.5±0.2	27.4±0.3
Lysine[a]	13.8±0.2	23.6±0.2
Methionine[a]	4.7±0.1	6.8±0.1
Phenylalanine[a]	11.9±0.2	14.9±0.2
Proline	11.8±0.2	8.7±0.2
Serine	5.7±0.1	9.9±0.1
Threonine[a]	9.6±0.1	6.9±0.1
Tryptophan[a]	3.4±0.1	4.3±0.1
Tyrosine	13.7±0.1	19.8±0.1
Valine[a]	7.7±0.1	8.5±0.1
EAA	82.2±0.7	118.6±1.0
TAA	164.7±1.4	216.1±2.2
(EAA/TAA)/%	49.9±0.8	54.9±0.8

[a]essential amino acid (EAA); TAA – total amino acid

Conclusion

Dry cell mass, Cr content in mycelia and the rate of Cr enrichment were taken as evaluating parameters for Cr enrichment in *P. cornucopiae* SD-01 in submerged culture on the basis of the orthogonal experimental design and single-factor tests. The optimum Cr concentrations had a positive effect on biomass production, Cr content, Cr-enriched rate, protein and amino acid biosynthesis of *P. cornucopiae* SD-01 in submerged culture. Thus, it is possible for *P. cornucopiae* SD-01 to produce the functionally Cr-enriched foodstuff or additives by commercial fermentation, and that the Cr-enriched mycelia represent a novel dietary source of bioavailable supplemental chromium.

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