Production of selenium-enriched rapeseed peptides

LIU Dachuan[1], ZHOU Junmei[2], ZHANG Hanjun[1]

[1.] *Wuhan Polytechnic University, 430023 Wuhan, China; Email: zhj@whpu.edu.cn*
[2.] *Wuhan Institute of Research & Design of State Administration for Grain Reserve, 430079 Wuhan, China*

Abstract

Research has been carried out on extracting Selenium-enriched rapeseed protein isolate (RPI). Based on single factor experiment, the extraction conditions had been optimized by orthogonal analysis. The optimum conditions for phytic acid and glucosinolate extraction are that the Selenium-enriched defatted rapeseed meal is extracted 3 times with a solid to solution ratio of 1:10 at 45°C for 60 min each at a pH of 4.0. The optimum extraction conditions for Selenium-enriched rapeseed protein isolate are that the meal is extracted 3 times with a solid to solution ratio of 1:12 at 50°C for 35 min each under pH12.0.

Enzyme hydrolysis for Selenium-enriched rapeseed protein isolate has been carried out. An alkaline enzyme, alcalase, was selected to hydrolyze the Selenium-enriched rapeseed protein isolate. The substrate (having a concentration of 3%) was digested with alcalase (180000U/g) for 7 hours at 50°C and a pH of 8.0. Under this condition, 32.51% of the substrate is hydrolyzed. The nitrogen recovery rate is 91.28%. The crude production is then decolorized with active carbon and desalinized, concentrated and lyophilized. Molecular weight of Selenium-enriched rapeseed peptide is lower 1500 Dalton.

Key words: Selenium-enriched rapeseed protein isolates,enzyme hydrolysis,Selenium-enriched rapeseed peptides

Introduction

Selenium is a trace element which can be used to cure many disease such as cancer, cardiovascular and eye disease. It is highly profitable and profoundly remarkable to develop selenium-enriched products. Rapeseeds absorb and enrich inorganic-Se from Se-enriched soil and transform to Se-Met and Se-Cys. Rapeseeds can accumulate as high as 10ppm and are good organic-Se resource.

In this research, we optimized the conditions for preparation of Se-enriched rapeseed protein isolate from defatted rapeseed meal and then optimized the conditions for preparation of peptide using enzyme hydrolysis.

Material and Methods

Raw material

Selenium-enriched rapeseed from En-shi city of Hubei province

Table 1 Main ingredients of stuff

ingredient	rapeseed	husked rapeseed	defatted meal
crude protein (N*6.25, dry basis,%)	25.19	28.57	39.04
ash content (%)	4.4	3.4	5.9
crude fat content (%)	37.53	42.39	0.48
moisture content (%)	7.91	6.30	8.51
tannin content (%)	2.52	2.71	3.62
phytic acid content (%)	2.38	2.72	4.02
glucosinolate (mg/g)	14.01	16.36	25.11
selenium (ppm)	2.21	2.53	3.30

Experiment

Phytic acid and glucosinolate were extracted from Selenium-enriched rapeseed defatted meal in the condition of pH5.0. The supernate was got off by centrifugating and the precipitate was the low-phytic acid rapeseed meal in which Selenium-enriched rapeseed protein isolate was prepared. The water was added in the meal in a certain proportion then adjusted pH to extract the protein effectively. After the mixed liquid was centrifugated, the above fluid was collected and settled by adding acid to pH4.0. The sediment is Selenium-enriched rapeseed protein isolate.

Selenium-enriched rapeseed protein peptide was prepared from protein isolate. Before hydrolyzing, there is a pretreatment on protein of dissolving in water, heating up on above 70°C and then 30minutes later, cooling to room temperature, adjusting pH, adding enzyme liquid, hydrolyzing several hours on constant temperature, heating at 80°C to destroy the enzyme activity, centrifugating and collecting the clear fluid, that is protein peptide liquid.

The effect factors of enzyme hydrolyzing are the choice of enzyme, the dose of addition, the substrate concentration and the time. First, according to the different effect of proteolytic enzyme A, B, C and D, one of was choiced to hydroze the

Selenium-enriched rapeseed protein isolate. Then the study of the other 3 factors carried on. In the single factor experiment, every factor has five levels, such as the dose of enzyme addition (3×10^4U/g, 6×10^4U/g, 9×10^4U/g, 12×10^4U/g, 15×10^4U/g), the substrate concentration (1%, 3%, 5%, 7%, 9%), the enzyme hydrolysis time (4h, 5h, 6h, 7h, 8h). When one factor levels are changed, the others are changeless. Which level of one factor was choiced lay on the degree of hydrolysis (DH) and the recovery rate of nitrogen. Based on the single factor experiment, the hydrolysis conditions would be optimized by orthogonal analysis. The method for determination of DH is formaldehyde titrating rapidly to free amino nitrogen, and the recovery rate of nitrogen is Kjeltec nitrogen analyzer (HE Zhao-fan & ZHANG Di-qing, 1997; WANG Zhao-ci, 2000).

Results and Discussion

In this experiment, the condition of extracting phytic acid and glucosinolate had been confirmed. That was pH 4.0, solid to solution ratio 1:10, temperature 45°C, 3 times and 60min every time. The condition of preparing protein isolate was pH12.0, solid to solution ratio 1:12, temperature 50°C, 3 times and 35min every time. Under the technics, the yield of Selenium-enriched rapeseed protein isolate was 26% and protein content was 83.37%.

Table 2 the main target of Selenium-enriched rapeseed protein isolate

ingredient	content
protein (N*6.25, dry basis,%)	83.37
crude fat content (%)	0.61
moisture content (%)	6.34
tannin content (%)	1.41
phytic acid content (%)	0.5
glucosinolate (mg/g)	0
selenium (ppm)	5.87

Selenium-enriched rapeseed protein isolate was a middle product, it had high protein content, low fat content, and it was pretreatment to have a very low anti-nutrition content, such as phytic acid, glucosinolate and tannin etc. because selenium element is integrated some amino acids, it almost had no lost and its content had high enrichment. This is a very good base for peptide preparation.

In a following part of this single factor experiment, first several kinds of enzyme were operated to choice a better efficiency to increase DH and the recovery rate of nitrogen, showing as the figure 1. Then as show below figure 2 to figure 4, enzyme addition, concentration of substrate and enzymehydrolysis time the 3 single factors were carried one by one to choice the best level. The best level as a midpoint, 3 levels every factor were done orthogonal analysis experiment as below table 2.

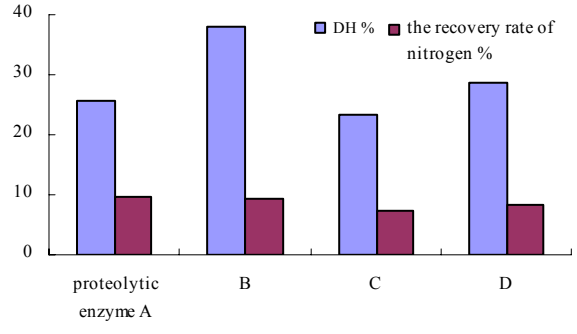

Fig.1 Effect of different enzymes on DH and the recovery rate of nitrogen solid to solution ratio1:20, the dose of enzyme3×10^4U/g, 50°C, 4h

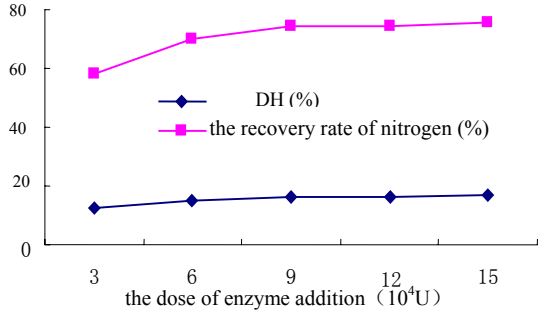

Fig.2 Effect of enzyme addition on DH and the recovery rate of nitrogen solid to solution ratio1:20, pH=8, 50°C, 4h

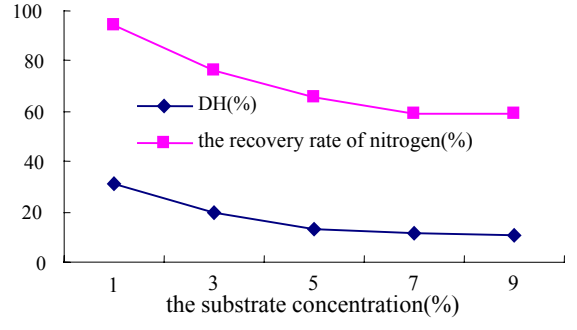

Fig.3 Effect of concentration of substrate on DH and the recovery rate of nitrogen
pH=8, the dose of enzyme addition9×10^4U/g, 50°C, 4h

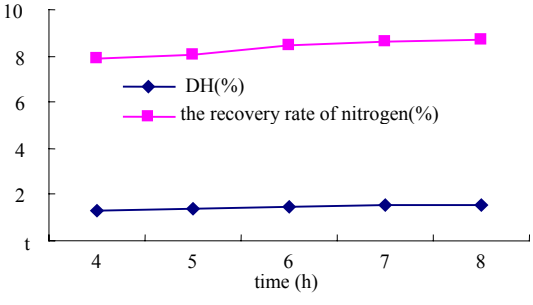

Fig.4 Effect of enzymehydrolysis time on DH and the recovery rate of nitrogen
pH=8, the dose of enzyme addition9×10^4U/g, 50°C

Table 2 result of orthogonal experiment

number	A dose of enzyme addition (10^4U/g)	B substrate concentration (%)	C time (h)	DH (%)	recovery rate of nitrogrn (%)
1	12	3	5	29.82	88.61
2	15	3	6	30.11	90.37
3	18	3	7	32.09	91.93
4	12	5	6	26.24	84.65
5	15	5	7	27.43	85.59
6	18	5	5	27.77	86.82
7	12	7	7	25.13	82.80
8	15	7	5	25.65	86.18
9	18	7	6	26.60	84.24
k1	27.063	30.673	27.747		
k2	27.730	27.147	27.650		
k3	28.820	25.793	28.217		
k1′	85.353	90.303	87.203		
k2′	87.380	85.687	86.420		
k3′	87.663	84.407	86.773		
R	1.757	4.880	0.567		
R′	2.310	5.896	0.783		

In the single factor of enzyme choice experiment, the proteolytic enzyme B had a higher DH and recovery rate of nitrogen than proteolytic enzymeA, C and D. So we should select the enzyme B to hydrolyze rapeseed protein for peptide. As figure2 show, along with the addition of proteolytic enzyme, the DH and nitrogen recovery rate were increased gradually. When the dose of enzyme was over 9×10^4U/g, the increase was a little slow, so we can select the dose 9×10^4U/g. From the figure 3, the DH and nitrogen recovery rate were declined obviously along with the increase of substrate concentration. If the addition of water is too high, the dose of protein will be too low correspondingly. Considering the cost is enhancive, so we can select the substrate concentration 3%. As the figure 4 show, along with the addition of hydrolysis time, the DH and nitrogen recovery rate were increased gradually. When the time was over 7h, the increase was a little slow, so we can select 7h as the best hydrolysis time.

From the table2, of the 3 effect factors, the effect of the substrate concentration is largest on rapeseed protein isolate hydrolysis, the addition dose of enzyme is taken second place, and the hydrolysis time is least. According to maximum variance analysis, the effect is B>A>C. According to the variance analysis of DH and the recovery rate of nitrogen, the remarkable effect factor is the substrate concentration. So the single factor tests and orthogonal experiments had been applied to optimize condition of enzymolysis as follows: pH8.0, substrate concentration is 3%, the time of enzymehydrolysis is 7 hours, temperature 50°C, and the dose of enzyme B is 18×10^4 U/g. Under the optimized condition, the drgree of hydrolysis (DH) is 32.51%, the recovery rate of nitrogen is 91.28%.

Refining

The hydrolysis product of Selenium-enriched rapeseed protein was yellowy fluid. It was dealt with pulverous active carbon to decolorize. The discoloring condition was pH3.0, the dose of active carbon 1%, heating temperature 70°C, the time 1h. Then filtrate when being hot, it was colorless. Whereafter the peptide liquid was desalinated with a dialysis bag which can intercept molecule weight 100. Finally the peptide was concentrated and was dried to be finished product. The ingredients of finished product are following:

Table 3 The main target of Selenium-enriched rapeseed protein peptides

ingredient	content
protein(N*6.25, dry basis,%)	87.11
crude fat content (%)	0.39
moisture content (%)	8.91
ash content (%)	4.79
NSI (%)	95.45
tannin content (%)	1.28
phytic acid content (%)	0.23
glucosinolate content (mg/g)	0
selenium (ppm)	6.02
solubility in TCA (%)	95.11

Conclusions

Selenium-enriched rapeseed husked and defatted meal was as a raw material to prepare rapeseed protein isolate that was pretreated to reduce its phytic acid and glucosinolate content. Rapeseed protein isolate was a middle product to prepare rapeseed peptide by enzyme hydrolysis. The composing ingredient of this peptide production is: protein content 87.11%, NSI 95.45%, solubility in TCA 95.11%, moisture content 8.91%, tannin content 1.28%, phytic acid content 0.23%, glucosinolate content 0, selenium content 6.02ppm. After determining, molecular weight distributing of peptide production is well, and it

almost all is small molecular peptide. The Selenium-enriched rapeseed protein peptide has lower 1500D average molecular weight. The rate of 926D is 78.28%, 675D is 18.89%, 233D is 2.9%.

References

Bigelow C C, (1967). On the Average Hydrophobicity of Proteins and the Relation Between it and Protein Structure, J Theoret Biol, **16**, 187-211
HE Zhao-fan, ZHANG Di-qing, (1997). Chemistry and determination technique of health food product, Bei-jing, China.
John, A.M., (1984). Selenium and the transplantable tumor, Agric. Food Chem., **32**, 437-438
LI Qiu-yue. (1987). General utilization of rapeseeds, Bei-jing, China.
Netto FM, Galeazzi AM. (1998). Production and Characterization of Enzymatic Hydrolysate From Soy Protein Isolate, Food Sci Technol-Leb, **31**, 624-631
Passuater, R.A., (1980). Selenium as Food and medicine, Keats publishing.
WANG Zhao-ci, (2000). Quality analysis of grain and oil product, Bei-jing, China.