Preparation of peptides hydrolyzed from rape pollen glutelins

HU Xiaobo[1], XUE Zhaohui[2], WU Moucheng[1*]

[1]College of Food Science and Technology. Huazhong Agricultural University. China.430070
[2]College of Agriculture and Bioengineering, Tianjin University, Tianjin. China.300072
Email: hxbxmg@mail.hzau.edu.cn; wumch98@mail.hzau.edu.cn

Abstract

Rape pollen stored more than 1 year was used as material in this study. A research on protein fractionation of rape pollen defatted and cell-fragmentated was performed. Rape pollen gluntelin was hydrolyzed with alcalase and the crude peptides of rape pollen were obtained. The scavenging effect of rape pollen glutelin and its hydrolysate on hydroxyl free radicals(·OH) in the deoxyribose-iron system was studied. The results showed that glutelins and albumins were found to be the predominant proteins in rape pollen,comprising 55.7% and 39.0% of total proteins, While globulins and prolamins were 3.2% and 2.1%,respectively.The optimum conditions of alcalase enzymatic hydrolysis have been determined by mono-factor analysis and response surface methodology as follows:pH =9,hydrolyzing temperature 50°C,enzyme concentration 1460U per gram of substrate, concertration of substrate 6%,hydrolyzing time 2h.In the antioxidation test, the inhibition rate ·OH of glutelins was 25.1%. After being hydrolyzed, the inhibition ratio of ·OH of glutelins peptide was singnificently increased to 70.0%.

Key words: Rape pollen, Enzymatic hydrolysis, Preparation

Introduction

Rapeseed is one of the most important oilseed crops cultivated in the world, thus rape pollen is the cheap high-yielding pollen. However, because the rape pollen has a special unpleasant flavor that can not be accepted by young people, it is not populared by consumers directly as a commodity for sale,leading to large bulks of storage. Protein content is as high as 20-25% in Rape pollen, which is comparable to soybeans and peas,suitable proportion of its amino acid composition, with higher contents of essential amino acids, much peptide of higher quality physiological activity is expected to be obtained after the enzymatic modification.

Alcalase is a microbial protease from the bacterium *Bacillus licheniformis* with endopeptidase activity, which can hydrolysis protein to be short peptides. Response surface methodology(RSM) was originally described by Box and Wilson(5)as being effective for responses that are influenced by many factors and their interactions.

The authors have carried out some preliminary work on the protein extracted from the rape pollen and enzymatic modification with the rape pollen stored one year as raw material. And the old rape pollen is much more conducive to breaking cell. The initial findings showed : All the nutrient contents of new rape pollen is not much different from the old one.The protein contents in rape pollen account for 22.4% after being defatted and cell-fragmentated. Glutenin contents account for more than half of the contents of total protein, however, glutelin is alkali - soluble protein and its bioavailability in human gastric environment is very low, thus peptide obtained following the enzymatic modification with glutelin used as raw material, which not only improve its own bioavailability, but also enhance biological activity. activity of anti-hydroxyl radical has increased markedly after enzymolysis.Reactive oxygen species including free radicals lead to lipid peroxidation in organism, and initiate. inflammation, cancer, aging, atherosclerosis and other origin of diseases. Further in-depth study is expected to obtain the rape pollen peptide of the physiologic function including higher anti-aging,anti-fatigue, immunological regulation, etc., and provide a new health care products for human beings, and is conducive to multiple utilization of resources of staple agricultural products such as rape,imcrease the added value of agricultural products,and is helpful to solve the "three rural issues".

Materials and Methods

Materials

Rape pollen Wuhan small Bee Pollen company
Alkaline proteinase companies :Denmark NOVO companies (activity of 146,000 u/mL)
Sephadex G-25 (Pharnacial), Bactiracin(Sigma),Glutathione(Reduced, Amresco)

All other chemicals were of analytical grade.

Methods

The determination of the protein content of each component of Rape Pollen after being defatted and cell- fragmentated

Use different solvents to extract albumin, globulin, gliadin and glutelin, concentrate the protein solution obtained, and then use micro-Kjeldahl method (Moucheng Wu,2002) to determine the protein content of each component.

Total nitrogen determination:

Total nitrogen was determined according to the micro-Kheldahl method, crude protein content was calculated using a conversion factor of 6.25.

Measurement of Degree of hydrolysis:

The degree of hydrolysis, defined as the ratio of amino nitrogen/total nitrogen(AN/TN), was calculated according to the methods of X. H. Zhao (8,9).

$$DH\% = AN(\text{amino nitrogen}) / TN(\text{total nitrogen}) \times 100\%$$

The AN, produced by hydrolyzing, was determined with formaldehyde titration procedure, While total nitrogen was determined according to the micro-Kheldahl method above(1.2.2).

Determination of the free amino acid content Ninhydrin chromatometry method (he sheng li, 2000)

The amino acid analysis of rape pollen protein isolates

HPLC analysis of amino acid composition.(Institute of the Chinese Academy of Agricultural Sciences, Wuhan Fuel Testing Center)

Determination of •OH inhibition ratio

•OH inhibition ratio is determined by Halliwell,etc (Halliwell et al.1987) and the method is to be improved. Referring to Halliwell (1987) method: The reagents added to a clean cuvette in order are as follows 0.4 mL of 50mmol·L^{-1} KH_2PO_4-KOH buffer (pH 7.5),0.1mL sample of a certain concentration, 0.1mL 1.04mmol·L^{-1} EDTA,0.1 L10mmol·$L^{-1}H_2O_2$, 0.1mL 60mmol·L^{-1}deoxyribose (not in the comparison),0.1mL 2mmol·L^{-1}VC,and 0.1mL 1mmol·L^{-1} FeCl3. every **c**uvette of the final volume is 1.0 mL. incubated at 37 ° C for one hour before removal, adding 1ml 25%HCl,terminating reaction,In addition,adding 1.0mL1%TBA solution to blend solution in each cuvette, then cooked in boiling water bath for 15 min. Cooling immediately after centrifugation (3000r/min). Measuring the absorbance at 532 nm, and calculating according to the following formula :

• OH inhibition rate (%) = [(A0 - A) / A0] × 100 (1-1)

where A0- not joined the absorption inhibitor •OH

A value-adding the inhibitor optical • OH

Preparation of rape pollen defatted and cell-fragmentated

After the impurities in rape pollen and the molded pollen grain being removed, grinding full,refrigerating at -10 degrees above freezing for 24 hours, stirring in the hot water with 80 °C,immediately cooled to 40 —45 °C and stirred at this temperature for extraction for 8 ~ 10 h.then freeze drying until rape pollen of which cell have been broken be obtained (Zhi Liu, 1989). Pollen obtained will be soaked with petroleum ether after the grinding, then placed in the fume cupboard for ventilation for 12h,recirculated and condensed in the water bath at 55 °C for 8h, filtrating, placed in the fume cupboard for ventilation for one hour, then vacuum dried at constant temperature of 45 °C.defatted pollen of which cell has been broken will be obtained.Pollen has been dried is put into the brown bottle, and placed in the shady place for reserve(Adnan et al., 2000).

Preparation of pollen protein isolates

The electrostatic repulsion of protein between particles in the electrostatic state is the smallest,therefore the solubility is minimal,The isoelectric points of proteins are different,thus adjusting the solution pH at or near the isoelectric point of a certain protein can be used for protein precipitation so as to achieve the purpose of purification, then extracting.

Nutritive value evaluation of pollen protein isolates

Evaluating essential amino acids (EAA) with the method of amino acid ratio coefficient in the non-biological evaluation method (Sheng-Tao Zhu and Wu Kun,1988). calculating the following index with the standard the World Health Organization (WHO) and UN Food and Agriculture Organization (FAO) proposed, aimed at the recommended values of the essential amino acids (E. C. Henley;J. M. Kuster, 1994)for the children aged 10-12 :amino acid ratio (ratio of amino acid, RAA).amino acid ratio coefficient (the ratio coefficient of amino acid.RC) and the amino acid ratio coefficient pm (Score of RC, SRC).Formula is as follows : RAA=the content of the essential acid in rape pollen/the RDA of the essential acid

RC=RAA/the average of RAA

CV=the standard deviation of RC/ the average of RC

SRC=100-CV*100

If the amino acid composition of food is consistent with the recommended values of amino acids, then RC = 1. RC>1 or RC<1 demonstrate that deviating from the amino acids mode.RC>1 demonstrate that the amino acids is relatively surplus,RC <1 demonstrate that this amino acids is relatively insufficient ; amino acid of which RC is the minimal is the first limited amino acid.

Enzymolysis reaction of alkali-soluble glutelin

Collocating alkali - soluble protein solution of a certain concentration,heating the solution to temperature of test set in waterbath,adjusting ph to the ph of test set,adding 0.25mL alkaline proteinase and stirring at lower rate.maintaining the stability of ph by adding diluted acid and alkali once every half hour.after 2 hour's Hydrolysis, enzymolysis solution is placed in the boiling water for the 10 min for the deactivation of protease, reaching the condition of metered volume after

cooling, filtrating, the filtrate is to be placed in the fridge for analysis。

Optimization of hydrolytic conditions

A three-factor central composite design was employed to examine the response,degree of hydrolysis (DH%) of glutelin by alcalase as changed with the independent variables, the substrate concentration, (%,X_1) temperature(°C,X_2)and pH value (X_3).A quadratic polynomial regression model was assumed for prediction the response. Every factor (Code X1 to X3) had three levels which corresponding to three code values.There were total 15 experiments conducted. In every experiment levels of the factors were arranged according to Table 1. The model proposed is described in Table2, under results and discussion, experimental data were analyzed for response surface regression for a quadratic polynomial model using SAS software (SAS Institute Inc.1990)

Table1 Design of factors levels in experiment

Factor	Code	Code value	Level
		+1	4
[S]/%	X1	0	5
		-1	6
		+1	40
T/°C	X2	0	50
		-1	60
		+1	8
pH	X3	0	9
		-1	10

Table2 Different levels of factors arranged in experiments

Test number	Code value of experiment		
	X1	X2	X3
1	-1	-1	0
2	-1	0	-1
3	-1	0	+1
4	-1	1	0
5	0	-1	-1
6	0	-1	+1
7	0	+1	-1
8	0	+1	+1
9	+1	-1	0
10	+1	0	-1
11	+1	0	+1
12	+1	+1	0
13	0	0	0
14	0	0	0
15	0	0	0

Results and discussuion

Distribution of protein component in rape pollen defatted and cell-fragmentated

Table 2-1 distribution of protein component in rape pollen

categpry	Distribution of proteins in rape pollen defatted and cell-fragmentated (%)	Distribution in total protein (%)
albumin	7.769	39.0
globulin	0.4310	3.20
alcohol-soluble protein	0.6300	2.10
alkali-soluble protein	11.11	55.7
total	22.42	100

The results can be got from Table 2-1: albumin and alkali - soluble protein are the major protein in pollen,accounting for more than 90% of pollen's total protein,which is the main component composing the pollen protein.in addition, it contains a small amount of globulin and prolamine accounting for approximately 5% of total protein.

Nutritive value evaluation of pollen protein isolates

The results can be got from Table 2-2, lys's and Tyr+Phe's amino acid ratio coefficient of albumin, globulin, glutelin, and Thr's amino acid ratio coefficient of glutelin are all more than one (RC> 1).it illustrates that these amino acids are relative surplus.As the result of Met ant Trp were not measured, these two amino acid were not taken into consideration in the process of evaluation. the RC of leucine in albumin and globulin are the lowest,thus leucine is the first restrictive amino

acid of albumin and globulin. the RC of isoleucine in alkali - soluble protein is the lowest, **so** isoleucine is the first restrictive amino acid of glutelin.Amino acid ratio coefficient (SRC) is to evaluate the protein quality by dispersion that all essential amino acids diverge from the amino acid model,SRC is highly correlated with the biological value and also is closer to biological value in number. it also indicates that from the table 2-2 the SRC of the glutelin is the highest, therefore its nutritional value is the best, and although the albumin is of the high content,its nutritional value is Almost not too much. alkali - soluble glutelin in human gastric acid environment is of very low bioavailability, therefore, we use glutelin as the experimental material for the follow-up experiments,and then enzymatic modification not only can increase bioavailability,but also increase biological activity.

Table 2-2 amino acid ratio of albumin, globulin, glutelin, amino acids Coefficient ratio and distribution of amino acid ratio coefficient (content of alcohol-soluble protein is little)

	Ile	Leu	Lys	Met+Cys	Trp	Tyr+Phe	Thr	Val	average	SRC
Ref value (10~12y)	2.80	4.40	4.40	2.20	0.90	2.20	2.80	2.50		
albumin										-0.20
RAA	0.100	0.093	0.825	0.068	—	0.286	0.343	0.152	0.267	
RC	0.375	0.348	3.090	0.255	—	1.071	1.285	0.569	1.000	
globulin										18.67
RAA	0.082	0.073	0.543	—	—	0.273	0.161	0.164	0.216	
RC	0.380	0.338	2.514	—	—	1.264	0.745	0.759	1.000	
glutelin										42.25
RAA	0.354	0.486	1.121	—	—	1.827	0.918	0.776	0.914	
RC	0.387	0.532	1.226	—	—	1.999	1.004	0.849	1.000	

Experimental results of enzymatic hydrolysis condition

Experiments are conducted in a random order for three repetition,the average of absorbance obtained in the experiment will be analyzed through SAS RSREG (Response Surface Regression) program, and response surface analysis graph and variance analysis table will be obtained.

Table 2-5 Parameters estimated in regression models (RSM analysis)

Parameter	a_0	a_1	a_2	a_3	a_{11}	a_{22}	a_{33}	a_{12}	a_{13}	a_{23}
Value in model about degree of hydrolysis	29.39	0.21	1.00	3.30	-4.05	1.46	-12.04	-3.54	1.08	-5.86

Table 2-6 The predicted value of regression model and experimental results

Response variable		Degree of hydrolysis	
[S]/%	T/°C	pH	%
6.20	49.8	8.69	17.8

Table 2-7 variance analysis of The regression equation

source of variance	Degree of freedom	Sum of square	FValue
Model	9	989.637	8.17*
Error	5	984.612	
Correct total	14	1051.537	
Linearly dependent coefficien	0.9364		

**$f_{0.01}(9,5)=10.2$; *$f_{0.05}(9,5)=4.8$

Results of 15 experiments were in table 3.Content of Hydrolysis Degree (DH) were used as response values in analysis of response surface regression(RSREG). The equation $DH(Y) = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3$ was used as regression model. The procedure RSREG of SAS also gave values of parameter estimated (Table 2-7). SAS RSREG procedures calculated using the regression equation coefficients (Table 2-3) on the basis of the mathematical analysis of the regression model, the results shown in table 2-4.Further analysis and regression model to estimate the value of more than f0.05 model F (9, 5).linear reached 0.9364 (Table 2-5),explain**ing** this regression equation to describe the relationship between the various factors and response,with all of its variable linear relationship between variables is notable that this method is reliable. The results of the regression model can be used to analyze and forecast. Alcalase endoprotease alkaline hydrolysis of insoluble protein substrate concentration, the optimal reaction conditions : 6%.pH 9.0, the temperature was 60 °C, compared with the end of 1460U/g protein,enzyme hydrolysis time 2h.

Studies on each pollen protein isolates and antioxidant activity of glutelin enzymolysis peptide.

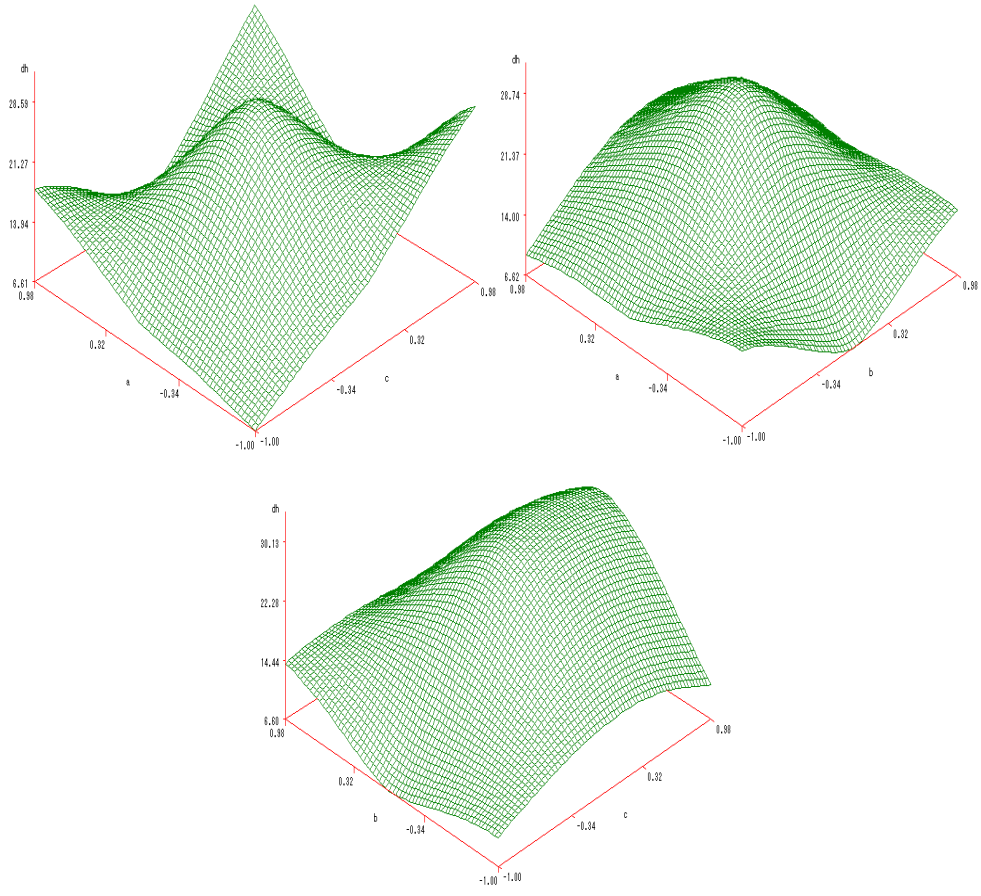

Fig.1 Response surface diagrams of Degree of hydrolysis

Biologically active peptide with low molecular weight**, having a strong antioxidant capacity,** will be formed after Pollen porotein is hydrolysed with protease.The results of. Table 2-8 show that the pollen alkaline protease rate of inhibition on OH is 25.08% before hydrolysis.While being hydrolysed it can got to 70%. It demonstrates that the activity of pollen alkaline protease improve significantly after hydrolysis.

Table 2-8 The hydroxyl radical inhibition rate of each pollen protein isolates and glutelin enzymolysis peptide. (%)

concentration (mg/ml)	2	2	2	2
Sample	albumin	globulin	glutelin	glutelin enzymolysis
The inhibition rate (%)	54.35	45.03	25.08	70.0

Conclusion

The results showed that glutelins and albumins were found to be the predominant proteins in rape pollen, comprising 55.7% and 39.0% of total proteins.While globulins and prolamins were 3.2% and 2.1%, respectively. The optimum conditions of alcalase enzymatic hydrolysis have been determined by mono-factor analysis and response surface methodology as follows:pH =9,hydrolyzing temperature 50°C,enzyme concentration 1460U per gram of substrate, concertration of substrate, 6%,hydrolyzing time 2h.In the antioxidation test,the inhibition rate ·OH of glutelins was 25.1%.After being hydrolyzed,the inhibition ratio of ·OH of glutelins was singnificently increased to 70.0%. It demonstrates that the activity of pollen alkaline protease improve significantly after hydrolysis.

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