Study on Glycosylation Modification Technology of Semen Euphorbiae Meal Protein

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Abstract: In order to improve the solubility, emulsification and foamability of the Semen Euphorbiaee, and to improve the utilization of the Semen Euphorbiae, this experiment used glucose as a sugar source and modified the Semen Euphorbiae protein by wet glycosylation. Using solubility as a judgment index, a single factor test was conducted to optimize the conditions for the glycosylation reaction of the golden gold meal protein. The results showed that the solubility and other functional properties of the golden gold meal protein obtained by glycosylation under the conditions of pH 10.5, temperature 83.5 ° C, and meal protein / glucose mass ratio 1: 1.5 were improved.

1 Introduction

Semen Euphorbiae is the dried mature seeds of Euphorbia lathyris L, which has the functions of expelling water retention by purgation, activating blood and eliminating mass. It is the main raw material for the production of Chinese medicine Fukeqianjin Tablets [1]. Semen Euphorbiae rich in oil and containing other chemical components such as protein, terpenoids, steroids and coumarins has the functions of removing inflammation and swelling, inducing diarrhea, resisting tumors, etc. [2] Semen Euphorbiae meal protein is an important by-product in the extraction process of Semen Euphorbiae, among which the content of crude protein is 32.2% [3-4]. At present, the studies on Semen Euphorbiae mainly focus on the chemical components of oil, terpenoids and coumarins, and there is very little research and utilization of protein.

Semen Euphorbiae meal protein contains many kinds of amino acids, which are mostly glutamic acid and aspartic acid [5-6]. With the glycosylation modification technology, Semen Euphorbiae meal protein is modified into lipoglycoside-bonded glycoprotein [7-8]. This technology helps improve the functional properties of protein, which can be better applied in traditional Chinese medicine treatment, clinical use, and drug research and development, providing support for the research technique and discovery status of Semen Euphorbiae meal protein.

2 MATERIALS AND INSTRUMENTS

Semen Euphorbiae, petroleum ether (30-60℃), glucose, coomassie brilliant blue G-250, standard bovine serum albumin, sodium lauryl sulfate, o-phthalaldehyde, sodium hydroxide solution, hydrochloric acid solution, etc. Electronic analytical balance, multi-functional centrifuge, UV1000 UV-vis spectrophotometer, DZF-6050 vacuum drying oven, etc.

3 EXPERIMENTAL METHODS

3.1.Preparation of Glycosylated Proteins

Weigh the Semen Euphorbiae meal protein to prepare the 1.2%w/v solution, which is adjusted to a pH of 11, stirred for 2 hours, mixed with glucose in proportion and stirred for an hour. Adjust the pH to the required value with 1mol/L HCl solution or 1mol/L NaoH solution, and keep stirring. Take 15 mL of the mixed solution and heat it in a water bath at a certain temperature. After the reaction is over, put it in ice water and cool it for 5 minutes before chilled storage.

3.2.Determination of DG (Degree of Graft) and Browning Degree

3.2.1.Determination of DG

The DG is determined by the o-Phthalaldehyde (OPA) method. Accurately weigh 40.0 mg of OPA, dissolve it with 1.0 mL of methanol, add 2.5 mL of 20% (w/w) sodium dodecyl sulfate (SDS), 25.0 mL of 0.1 mol/L borax, and 100 μL of β-mercaptoethanol after the

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dissolution is complete, and finally make the solution reach a constant volume of 50 mL with distilled water. During the process of determination, put 4.0mL of OPA reagent into a test tube, add 200μL of cooled reaction solution of Semen Euphorbiae meal protein and glucose, mix it well and let it react in a 35℃ water bath for 2 minutes, and finally determine the absorbance ratio At at 340nm. The blank sample is prepared by adding 200μL of water into 4.0mL of OPA reagent and the absorbance ratio is A0 at 340nm. The DG is calculated according to the following formula.

$$DG(\%) = \frac{A_0 - A_t}{A_0} \times 100$$

In this formula, DG - Degree of Graft (%)

A0 - the absorbance ratio of the blank sample is A0 at 340nm.

At - the absorbance ratio is At at 340nm after 2 minutes of reaction.

3.2.2.Determination of Browning Degree

Accurately take 1.0mL of grafting reactant of Semen Euphorbiae meal protein and glucose, mix it with 5.0mL of 0.1%(w/w) SDS solution, and determine the absorbance ratio A420 at 420nm. Taking SDS solution as a blank, the absorbance reflects the browning degree.

3.3.Study on the Factors Affecting the Solubility of Meal Protein

The protein is modified with wet glycosylation. The main factors include pH, temperature, the mass ratio of Semen Euphorbiae meal protein/glucose. The solubility is used as a criterion to determine the optimal reaction conditions for preparing covalent complexes, so as to optimize the modification technology.

3.3.1.The effect of pH on Complexes

With the reaction temperature fixed at 100 ℃ and reaction duration of 20 minutes, Semen Euphorbiae meal protein and glucose are mixed with the mass ratio of 1:1. Prepare covalent complexes by adjusting pH to five levels respectively including 8, 9, 10, 11 and 12 according to the above-mentioned method in 2.2, and then determine the solubility.

3.3.2.The Effect of Mass Ratio of Meal Protein/glucose on Complexes

The reaction temperature is fixed at 100 ℃, reaction duration of 20 minutes and reaction pH the optimal value. Prepare covalent complexes by controlling the mass ratio of Semen Euphorbiae meal protein/glucose to five levels respectively including 1:1, 1:2, 2:1, 1:3 and 3:1 according to the above-mentioned method in 1.2.2, and then determine the solubility.

3.3.3.The Effect of Reaction Temperature on Complexes

With the reaction duration fixed at 20 minutes, reaction pH and mass ratio of Semen Euphorbiae meal protein/glucose fixed at the optimal value, and reaction temperature controlled at five levels respectively including 60, 70, 80, 90 and 100℃, prepare covalent complexes according to the above-mentioned method in 1.2.2, and then determine the solubility.

3.4.Experimental Design Optimization with Response Surface Method

According to the results of the single factor experiment, using the Box-Benhnken principle, taking pH value (X1), protein-glucose mass ratio (X2), temperature (X3) as independent variables, and solubility as the response value, a three factors-levels response surface experimental design is carried out.

3.5.Comparison of Properties for Semen Euphorbiae Meal Protein Before and After Glycosylation

3.5.1.Solubility

Weigh a certain amount of Semen Euphorbiae meal protein and covalent complexes, which are then prepared into a 1.2% (w/v) solution. Centrifugalize it for 20 minutes (7000r/min). Then determine the protein content in the solution with the coomassie brilliant blue G-250 method.

Solubility S(%)=the protein mass in supernatants(g)/the total protein mass in the sample(g)*100%

3.5.2.Foaming Property

Weigh a certain amount of Semen Euphorbiae meal protein and covalent complexes, which are then prepared into a 1.2% (w/v) solution. Let the solution stand for 1 minute (7000r/min). Immediately record the total volume of the foam V0(mL). After letting the solution stand for 10 minutes, record the total volume of the foam V10(mL) at this time, and the foam stability.

Foaming Properties FA=(V0/20)*100%

Foaming Stability FS=(V10/V0)*100%

In the formula, V0—the total volume of the foam after the standing; V10—the total volume of the foam after the solution stands for 10 minutes.

3.5.3.Emulsifying Property

Weigh a certain amount of Semen Euphorbiae meal protein and covalent complexes, which are then prepared into a 1.2% (w/v) solution. Put 15mL of protein solution and 5mL of soybean oil into the test tube, and let it stand for 1 minute. Then pipette 0.1mL of the solution from the bottom into another test tube, and add 10mL of 0.1% SDS solution. Take SDS solution as the blank solution, and

determine its absorbance at 500nm. The absorbance at 0min is the emulsifying property EA.

Emulsification Stability ES= (A0* A10)/ (A0- A10) *100%

In the formula, A0—the liquid absorbance value after the standing; A10—the liquid absorbance value after the liquid stands for 10 minutes.

4 RESULTS AND ANALYSIS

4.1.Technology Optimization of Meal Protein Glycosylation Modification

4.1.1.Results and Analysis of Single Factor Test

In this experiment, pH value, protein-glucose mass ratio and temperature are the influential factors, the solubility is the criterion for determination, and a single factor test is carried out to provide basis for the optimal experimental conditions and study different results of Semen Euphorbiae meal protein glycosylation modification under different conditions.

As the pH value continues to rise, the solubility of the protein keeps growing. When the pH value is 10, the solubility of the protein reaches the peak. And as the pH continues to rise, the solubility of the protein starts to decline constantly.

The effect of different Semen Euphorbiae meal protein/glucose mass ratios on solubility of meal protein and covalent complexes can be seen that with the use of glucose increases, solubility of the protein keeps growing. When the Semen Euphorbiae meal protein/glucose mass ratio is 1:1, solubility of the protein reaches the peak.

As the temperature continues to rise, solubility of the protein keeps growing. When the temperature is 80℃, solubility of the protein reaches the peak. As the temperature keeps increasing, solubility of the protein gradually decreases.

4.1.2.Results and Analysis of Tests with the Response Surface Method

A three factors-levels response surface experimental design test was designed with pH value, Semen Euphorbiae meal protein/glucose mass ratio and reaction temperature as the independent variables, and the solubility of the glycosylation products of Semen Euphorbiae meal protein as the response value. T

The ANOVA results of regression models can be seen that F-value is 19.00 and P-value is 0.0004, indicating that the regression model is highly significant and lack of fit of the model is not significant. R2=0.9576 R2Adj=0.9208, indicating that the regression equation of the test has a high degree of fitting, which can directly reflect the relationship between the reaction value and various factors and has a great statistical impact. Among these factors, reaction pH, quadratic term (B2, C2) of the reaction temperature, the Semen Euphorbiae meal protein and glucose mass ratio, and linear term (A, B) of reaction pH have extremely significant effects on solubility ($P<0.01$). Quadratic term (A2) of the Semen Euphorbiae meal protein and glucose mass ratio, the interaction item (AC) of the Semen Euphorbiae meal protein and glucose mass ratio, and the reaction temperature (C) have significant effects on solubility. The regression equation is obtained as follows by using Design Expert 11 software to perform quadratic polynomial regression fitting on the testing data:

$$Y = 31.42 - 1.11A - 1.50B + 1.83C + 0.17AB - 1.06AC - 0.30BC - 1.34A^2 - 2.07B^2 - 3.48C^2$$

According to the regression equation above, the optimal condition for glycosylation modification technology of Semen Euphorbiae meal protein is: the reaction pH value 10.62, Semen Euphorbiae meal protein/glucose ratio 1:1.46, and reaction temperature 83.57 ℃. The theoretical value of solubility of the covalent complex obtained under this condition is as high as 32.436%.

The response surface three-dimensional diagram and contour diagram obtained through the Design Expert 11 software show that the interaction between Semen Euphorbiae meal protein/glucose ratio and reaction temperature has a significant effect on the solubility. The results of ANOVA analysis are consistent with this result.

By means of model analysis, the optimal conditions for Semen Euphorbiae meal protein glycosylation modification are: pH value 10.5, Semen Euphorbiae meal protein/glucose ratio 1:1.5, reaction temperature 83.57℃, and the solubility of the covalent complex 32.43%. Considering the convenience of actual operation, the final determination of conditions for protein glycosylation modification technology is: reaction pH value 10.6, Semen Euphorbiae meal protein/glucose ratio 1:1.4, and reaction temperature 83.5 ℃. The verification test is repeated for three times and the solubility of the glycosylated protein is 32.9%, which shows that the regression model can match the actual situation and the prediction model is correct. Therefore, it is feasible to optimize the process conditions for Semen Euphorbiae meal protein glycosylation modification by the response surface method, which provides a basis for the research of Semen Euphorbiae meal protein.

4.2.Functional Property Determination of Meal Protein Before and After Modification

The covalent complexes are prepared under the optimal conditions. And the solubility of the meal protein before modification and functional properties of the covalent complexes are determined. The results are as shown in Table 3-1.

Table 3-1 Functional Properties of the Protein Before and After Modification

	Solubility %	Emulsifying property	Emulsifying Stability %	Foaming Property	Foaming Stability %
Meal protein	14.62	0.205	25.24	116.82	57.65
Covalent complex	32.43	0.620	35.86	147.91	61.64

4.2.1.Analysis of Solubility

From the table above, it can be seen that functional properties of the modified meal protein need to be improved. Before modification, solubility of meal protein is 14.62%, but after modification, the solubility increases to 17.81%. That means the solubility of meal protein after glycosylation modification is significantly improved, which may be due to the complex produced by combining protein and glucose. Glucose is polyhydroxyaldehyde, and hydrophilic hydroxyl groups are introduced with the protein, which increases the number of hydroxyl groups. A hydration layer is thus formed on the surface of the protein, and the solubility of the protein is improved. It is because the hydrophilic group is introduced into the amino acid chain to increase its hydrophilicity.

4.2.2.Analysis of Emulsifying Property and Emulsifying Stability

The emulsifying property of Semen Euphorbiae meal protein after modification is 0.205 and 0.620 respectively, and the emulsifying stability increases by 10.62%. The improved emulsifying property of the modified meal protein may be due to the introduction of hydrophilic hydroxyl groups on amino acid chains, which enables a part of the glycosylated protein to be adsorbed on the oil-water interface, thereby reducing the interfacial tension and improving the emulsifying property of the meal protein. In addition, the covalent complex molecules adsorbed on the oil-water interface have a larger molecular steric hindrance effect, which can prevent the generation of more droplets, promote the formation of fine emulsion particles, and the resulting emulsion system is more stable and the emulsifying stability is improved.

4.2.3.Analysis of Foaming Property and Foaming Stability

The foaming property of proteins before modification is 116.82 and that after modification is 147.91, with the foaming stability improved by 3.99%. The improved solubility of Semen Euphorbiae meal protein enhances the foaming capability of meal proteins, which helps the protein be diffused to the liquid surface more effectively and enhances the foaming capability of covalent complexes. In addition, as the solubility of Semen Euphorbiae meal protein increases and the protein is dissolved in the liquid, the sols concentration of the protein decreases, resulting in a decrease in its viscosity, an increase in foaming property, and an increase in foaming stability.

5 Conclusion

From the experimental results, it can be seen that the actual optimal parameters for the modification technology of Semen Euphorbiae meal protein with wet glycosylation is: pH value 10.5, temperature 83.5℃, Semen Euphorbiae meal protein/glucose mass ratio 1:1.5, and solubility 32.97%, an increase of 0.54% compared with the theoretical value of solubility (32.43%). The experiment has proved that the glycosylation technology of proteins can effectively improve the solubility, emulsifying property and foaming property of Semen Euphorbiae meal protein.

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