Research Article
The Effect of Glycosylation on the Functional Properties of Rice Protein

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Abstract: Due to its relatively low solubility, emulsibility and foamability, rice protein is restricted in the processing and utilization. The experiment showed that in the modified combination of optimal glycosylation obtained by the orthogonal method, its composite solubility increased from 3.43 to 32.75%, emulsibility increased from 33.85 to 48.96% and foamability increased from 16.9 to 30.9%. It indicated that glycosylation could effectively improve the functional properties of rice protein like solubility, emulsibility and foamability and contribute to the further processing and utilization of rice protein, thus laying good theoretical foundation for further study.

Keywords: Emulsibility, foamability, glycosylation, rice protein, solubility

INTRODUCTION

China is rich in rice resources. The rice processing industry is a traditional sunrise industry existing with human beings. No matter how advanced the science and technology are in the future, the rice processing industry can not be eliminated, but only improved and developed. Its byproduct also contains a lot of rice protein. Rice protein is an internationally recognized quality plant protein resource with high biological value (BV = 77) (Chen and Yao, 2002). Its amino acid composition comply with the ideal model recommended by the WHO/ FAO, in which methionine is high in content, which can not be compared by other edible plant proteins. In addition to the health care function, it has the characteristics of safety and low sensitivity and therefore is suitable as nutritional and health food for infants and special populations. At present, domestic and international research institutions attach great importance to the in-depth study of the rice protein and development of related products.

With the continuous improvement of the awareness of its value, rice protein has been developed into a product with high added value, such as rice protein foaming powder, rice protein nutritional powder, active beverage and resistance protein and can also be used as food additives, such as nutrition enhancer, ice cream and infant food (Kang and Wang, 2006). But for its poor solubility, it is generally only used as animal feed, resulting in a serious waste of resources and very limited use of rice protein in food. Generally, its functional properties are improved through the rice protein modification (Hamada, 1989), mainly including acidification (Naotoshi, 1985a, b), phosphorylation (Mathis, 1984; Frank, 1987), esterification (Mitra and Matsumoto, 1981), succinylation and glycosylation.

The purpose of this study is to achieve effective improvement and full utilization of the functional properties of rice protein, solve the technical problem of development of hydrophobic plant protein as functional food ingredient through the Maillard reaction (i.e., rice protein glycosylation) so that the rice protein low sensitivity and high nutritional properties are fully reflected and played.

MATERIALS AND METHODS

Rice protein, glucose, sucrose, D-xylose, pH test paper, accelerator, boric acid, cooking oil, distilled water, Sodium Dodecyl Sulfate (SDS). Centrifuge tube, pipette, beaker, volumetric flask, Kjeldahl flask, automatic Kjeldahl azotometer (KDY-9830), incubator (DH-360), electric heated water bath (DSY-Z-8), desktop electric centrifuge (TDL80-213), electric furnace (KD-9811), constant temperature magnetic stirrer (90-2), high-speed dispersion homogenizer (FA25), freeze dryer (LGJ-10).

Rice protein extraction (protease extraction): Rice → grinding → diluted   alkali   extraction →

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Table 1: Orthogonal experimental factors

Reaction time A (h)	glycosyl donor B	Reaction temperature C (°C)	pH	D
1	2	glucose	20	8
2	10	D-xylose	40	9.5
3	18	sucrose	60	11

Three orthogonal parallel experiments were performed with the solubility of the composite product of the glycosylated rice protein as the indicator to obtain the optimal solution through intuitive analysis

centrifugation → protein liquid → amylase hydrolysis → enzyme inactivation → centrifugation → precipitation → freeze drying → rice protein

Single factor experiment of glycosylation modified rice protein: Different reaction time, glycosyl donors, reaction temperatures and pH values were selected with solubility as indicators for univariate analysis to determine an optimal range of glycosylation modification.

Determination of the optimal solution of glycosylation modified rice protein (with L9 (34) orthogonal table, (Table 1):
Determination of solubility of rice protein: Dissolve 1g rice protein in distilled water to prepare the solution with the pH value of 9 (with 1mol/L NaOH and 1 mol/L HCl), take 100 mL, magnetically stir for 10 min, centrifuge for 10 min at 4000 r/min, take 1 mL supernatant and measure the nitrogen content of the supernatant with Kjeldahl azotometer. The solubility is calculated upon the equation: solubility = (nitrogen content of the supernatant/total nitrogen content) * 100%.

Determination of emulsibility of rice protein: Weigh 1 g rice protein and dissolve in distilled water to prepare the solution with the pH value of 9, stir and add 20 mL blend oil, add 5 mL 0.1% SDS solution, homogenate in the high-speed dispersion homogenizer for 1 min at 10000 r/min, draw 10 mL and centrifuge for 5 min at 4000 r/min. The solubility is calculated upon the equation: emulsifiability (%) = height of emulsion layer in centrifuge tube/total height of liquid in centrifuge tube * 100%.

Determination of foamability of rice protein: Precisely weigh 0.2 g rice protein and dissolve in 50 mL distilled water to prepare the solution with the pH value of 9, homogenate in the high-speed dispersion homogenizer for 1 min at 10000 r/min and weigh the initial foam height. The solubility is calculated upon the equation: foamability (foaming capacity, FC) = (foam height/total liquid height) *100%.

Determination of the functional properties of the optimal glycosylated product: A large number of glycosylated protein products were prepared with the best solution obtained from the orthogonal test and freeze-dried. The functional properties of the composite product were determined according to the above experimental method.

Comparison of the functional properties of rice protein before and after modification: Significant comparison of the solubility, emulsifiability and foamability of rice protein was made before and after modification.

Data analysis: Comparison between treatments was made using One-way ANOVA. Data were analyzed using Minitab Statistical Analysis Software, version 15. At least three replications for each material were used for these analyses. Graphical presentations were prepared based on the mean values using Microsoft Excel (2003 version).

RESULTS AND DISCUSSION

Effect of reaction time on composite solubility: Modification experiments were performed at 0, 4, 8, 12, 16 and 20h, respectively with solubility as the indicator. Other reaction conditions: temperature of 40°C, mass ratio of rice protein to glucose of 1:6, pH value of 9.5.

Figure 1 displays that the longer the reaction time, the higher the glycosylation of rice protein and that when the reaction time is 16-20 h, the solubility of glycosylated protein product will be increased gradually, possibly because the glycosylated rice protein reaches saturation and solubility of 28-30%.

Effect of glycosyl donor on composite solubility: Modification experiments were performed with glucose, D-xylose and sucrose respectively with solubility as the indicator. Other reaction conditions: temperature of 40°C, mass ratio of rice protein to glucose of 1:6, pH value of 9.5, reaction time of 10 h.

It can be seen from the results of Fig. 2 that glucose has the maximum effect on glycosylated product solubility of nearly 35% and D-xylose as the minimum effect on glycosylated product solubility of nearly 20%.
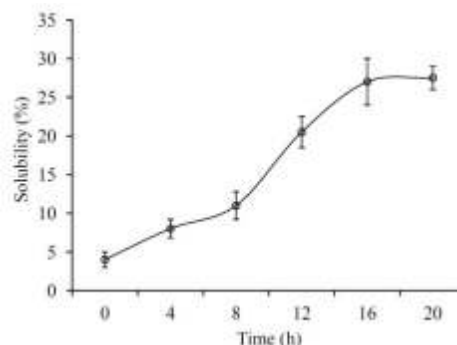

Fig. 1: Effect of reaction time on composite solubility

Table 2: Orthogonal experimental arrangement and experimental data

| Experiment No.	Factor solution	A(h)	B	C(°C)	D	Solubility (%)
1	$A_1B_1C_1D_1$	2	Glucose	20	8	13.21±0.65
2	$A_1B_2C_2D_2$	2	D-xylose	40	9.5	23.24±0.43
3	$A_1B_3C_3D_3$	2	Sucrose	60	11	17.23±0.97
4	$A_2B_1C_2D_3$	10	Glucose	40	11	31.24±0.56
5	$A_2B_2C_3D_1$	10	D-xylose	60	8	23.37±0.62
6	$A_2B_3C_1D_2$	10	Sucrose	20	9.5	25.22±0.48
7	$A_3B_1C_3D_2$	18	Glucose	60	9.5	32.31±0.63
8	$A_3B_2C_1D_3$	18	D-xylose	20	11	24.26±0.57
9	$A_3B_3C_2D_1$	18	Sucrose	40	8	27.89±0.78
Sum of results of level 1		53.679	76.761	62.691	64.470	
Sum of results of level 2		79.830	70.869	82.371	80.769	
Sum of results of level 3		84.459	70.341	72.909	72.729	
Mean 1		17.893	25.587	20.897	21.490	
Mean 2		26.610	23.623	27.457	26.923	
Mean 3		28.153	23.447	24.303	24.243	
Extreme difference		10.260	2.140	6.560	5.433	
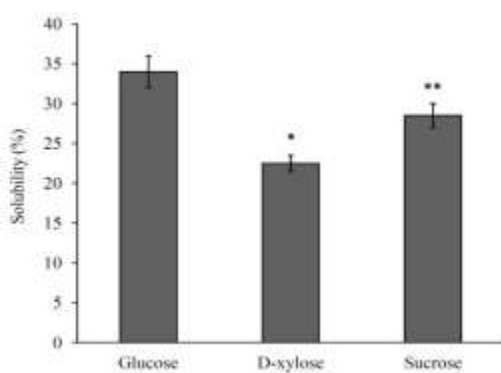
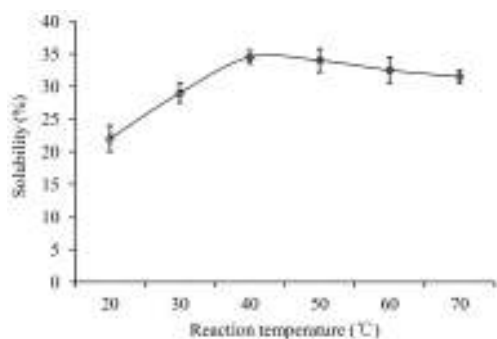Fig. 2: Effect of glycosyl donor on composite solubility

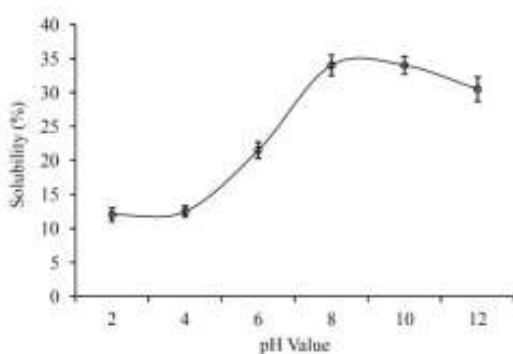Fig. 3: Effect of reaction temperature on composite solubility

Fig. 4: Effect of pH value on composite solubility

Effect of reaction temperature on composite solubility: Modification experiments were performed with the mass ratio of rice protein to glucose at 20, 30, 40, 50, 60 and 70°C, (heated in water bath) respectively with solubility as the indicator. Other reaction conditions: pH value of 9.5, reaction time of 10 h, mass ratio of rice protein to glucose of 1:6.

It can be seen from the results of Fig. 3 that after the reaction temperature reaches over 40°C, the modified protein increases gradually with the temperature and the solubility increases to some degree, not drastically. Even after the reaction temperature reaches over 50°C, the solubility decreases gradually. This was because some of the modified protein has thermal denaturation with the increase of temperature and develops into the insoluble protein so that the solubility can not increase significantly and even decrease. Considering the modified efficiency, the reaction temperature should be 40°C.

Effect of pH value on composite solubility: Modification experiments were performed with the pH value of 2, 4, 6, 8, 10 and 12, respectively with solubility as the indicator. Other reaction conditions: temperature of 40°C, mass ratio of rice protein to glucose of 1:6, reaction time of 10 h.

It can be seen from the results of Fig. 4 that when the pH value is 9, the inflection point occurs and the solubility of modified protein is highest.

Determination of the optimal solution of glycosylation modified rice protein (Table 2): Intuitive analysis was used to compare the R value of factors in the extreme difference analysis table. It can be seen that the reaction time is the most important influencing factor. The primary and secondary influencing factors in descending order are as follows: reaction time>reaction temperature> pH value>glycosyl donor. According to the analytical result of R value extreme difference, the optimal level combination of various factors was A3B1C2D2. The above study
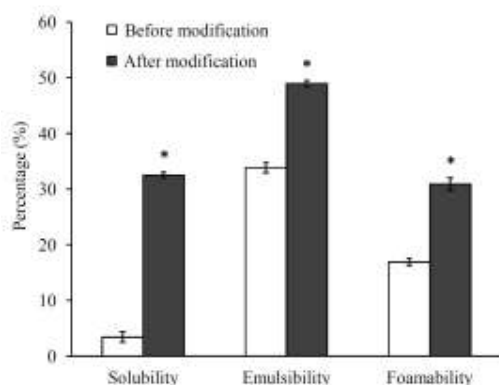
Fig. 5: Comparison of the functional properties of rice protein before and after modification *, $p < 0.05$ compared with rice protein before glycosylation modification

showed that the optimal process of rice protein glycosylation is as follow: reaction time of 18 h, reaction temperature of 40°C, pH value of 9.5 and glycosyl donor of glucose.

Determination of the functional properties of rice protein: The solubility of rice protein is not very good mainly because rice protein contains 57-90% of alkali soluble glutenin, which is formed by many macromolecular fragments via disulfide bonds and cross linked with each other and agglomerated. The clear water-soluble protein accounted for only 2-5% of the rice protein.

Emulsification includes emulsifying activity and emulsion stability. Emulsification is one of the important functions of the protein. Each protein has a certain molecular composition and specific spatial structure and its emulsifying property is closely related to the hydrophobicity of molecular surface. PH can change the charged nature of the protein and charge distribution, change the molecular spatial conformation and improve solubility, emulsifiability, formability and other physical and chemical functions of the protein (Wu *et al.*, 2007; Wang and Yao, 2005).

A study showed that the formability of rice protein increases with the increase of protein level (Tang *et al.*, 2003). To obtain the optimal formability, the solubility and hydrophobicity should be taken into account to strike the sound balance between hydrophilicity and hydrophobicity.

Determination of the functional properties of optimal glycoslation product: A large number of glycosylated protein products were prepared with the best solution and freeze-dried.

Comparison of the functional properties of rice protein before and after modification: It can be seen from Fig. 5 that solubility increased from 3.43 to 32.75%, emulsibility increased from 33.85 to 48.96%

and foamability increased from 16.9 to 30.9%. The change of solubility was the greatest and improved by nearly 10 times. And the increase of solubility is the most important in all functional properties in deep processing and utilization.

CONCLUSION

From this experiment, it is concluded that:

- During the glycosylation of rice protein, the longer the reaction time, the higher the glycosylation of rice protein. When the reaction time is over 16 h, the glycosylated rice protein reaches saturation and the increase rate slows down. Different glycosyl donors have different solubility, in which glucose has the maximal effect on the solubility of glycosylation product. After the reaction temperature reaches over 40°C, the composite solubility increases slowly. Even after the reaction temperature reaches over 50°C, the solubility decreases slowly. When the pH value is 9, the inflection point occurs and the solubility of modified protein is highest.

- For selection of optimal solution of glycosylated protein, the reaction time is the most important influencing factor. The primary and secondary influencing factors in descending order are as follows: reaction time> reaction temperature>pH value>glycosyl donor and the optimal solution is as follow: reaction time of 18 h, reaction temperature of 40°C, pH value of 9.5 and glycosyl donor of glucose.

- It can be seen from the experiment that due to its relatively low solubility, emulsibility and foamability, rice protein is restricted in the processing and utilization. After glycosylation modification, its solubility increased from 3.43 to 32.75%, emulsibility increased from 33.85 to 48.96% and foamability increased from 16.9 to 30.9%.

Most of domestic studies on improving the functional areas of the hydrophobic plant protein are carried out for soy protein. For cereal protein with urgent demand for improvement of its solubilization, especially for rice protein, there is basically no study in the technical field. The purpose of this study is to improve the functional properties of protein through controlling the Maillard reaction conditions, analyze the relationship between the improvement of the functional properties and influencing factors of reaction, achieve effective improvement and full utilization of the functional properties of rice protein, solve the technical problem of development of hydrophobic plant protein as functional food ingredient so that the rice protein low sensitivity and high nutritional properties are fully

reflected and played. It can be seen from the experiment that due to its relatively low solubility, emulsibility and foamability, rice protein is restricted in the processing and utilization. After glycosylation modification, its solubility increased from 3.43 to 32.75%, emulsibility increased from 33.85 to 48.96% and foamability increased from 16.9 to 30.9%. It is believed that the rice protein will have very broad prospects through the in-depth study and extended application. How to choose the glycosyl donor as well as how to improve the experimental method to increase changes of functional properties will be investigated in future studies.

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REFERENCES

Chen, J.W. and H.Y. Yao, 2002. Rice protein: Its development and applications. Sci. Tech. Food Ind., 23(6): 87-89.

Frank, A.W., 1987. Non-enzyme methods for the phosphorylation of proteins. Phosphor. Russ. Sulfur., 29: 297-315.

Hamada, J.S., 1989. Preparation and functional properties of enzymatically deaminated soy proteins. Food Sci., 54: 598-601.

Kang, Y.L. and Z.C. Wang, 2006. Research status of rice bran protein. Cereals Oils, 3: 22-24.

Mathis, G., 1984. Chemical phosphorylation of food proteins: An overview and a prospectus. Agric. Food. Chem., 32: 699-705.

Mitra, T. and H. Matsumoto, 1981. Flow properties of aqueous gluten methyl ester dispersions. Cereal Chem., 58: 57.

Naotoshi, M., 1985a. Conformational changes and functional properties of acid-modified soy proteins. Agric. Biol. Chem., 49(5): 1251-1256.

Naotoshi, M., 1985b. Polymerization of deaminated peptide fragments obtained with the mild acid hydrolysis of ovalbumin. Agric. Food. Chem., 33: 738-742.

Tang, S.H., N.S. Hettiarachchy and R. Horax, 2003. Physicochemical properties and functionality of rice bran protein hydrolazate prepared from heat-stabilized defatted rice bran with the aid of enzyme. J. Food Sci., 68(1): 152-157.

Wang, Z.C. and H.Y. Yao, 2005. Emulsifying properties of rice protein isolate. Food Sci., 26(2): 43-45.

Wu, J., W.W. Zheng, W.X. Zhao, D.D. Ren and C.L. Li, 2007. Optimization of emulsification activities of rice residues hydrolyzed protein-malt dextrin maillard conjugation by response surface methodology. Food Sci., 28(10): 155-158.