Optimization of the biological processing of rice dregs into nutritional peptides with the aid of trypsin

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Abstract A protein hydrolysate was prepared from rice dregs (RD) using trypsin as a suitable protease. The hydrolysis conditions were optimized with response surface methodology, and then a mathematical model was developed to demonstrate the effect of each process parameter on the degree of hydrolysis (DH) and recovery of protein (RP). A hydrolysate with relatively high RP (75.81%) and low DH (6.95) was obtained from RD by hydrolyzing for 2.4 h at pH 7.6 52.8°C with a enzyme/RD ratio of 0.89:1000 (w/w) and RD/water level of 0.22 g/mL. The dried hydrolysate was low molecular weight peptides (predominantly <1,000 Da) and it possessed good solubility at various pH levels. Result of amino acid analysis revealed that the hydrolysate was considerably enriched in essential amino acids. Thus, the protein hydrolysate has a potential to be an excellent protein ingredient as a balanced milk replacer.

Keywords Rice dregs protein · Enzymatic proteolysis · Response surface methodology · Nutritional properties

Introduction

RD is a major by-product of rice syrup production with an annual production of over 20 thousand tons from the south of China. RD retains nearly all the rice protein and has a protein content of more than 60% (dry matter basis), which is higher than soybean and comparable to fish meal. Rice protein is a valuable nutritional plant protein due to its ideal amino acid profile and beneficial for infants and seniors who are intolerant to cow's milk proteins for its properties of hypoallergenicity (Helm and Burks 1996). However, RD protein has the disadvantage of poor water solubility resulting from the denaturing heat of the rice syrup production process, which limited its application in food industry. The chemical or enzymatic modification for the production of rice protein may be an effective means to overcome this problem with retaining functional characteristics of rice protein.

Due to its simply processes, alkali dissolution followed by acid precipitation is the most common method to prepare the rice protein (Jiamyangyuen et al. 2005). But the process tends to be difficult to control and a number of toxic compounds such as lysinoalanine could be formed by side-reactions. Fortunately, controlled enzymatic hydrolysis of protein produces a series of small polypeptides, which could modify and improve the functional characteristics of proteins (Haard 2001). Enzymatic hydrolysis is an attractive means for it is performed under mild conditions of pH (6–8) and temperature (40–60°C). This could obtain the desired functional properties of food proteins and minimize the undesired side reactions without impairing the nutritional value of the protein (Clemente 2000).

Previous studies have shown that rice protein can be effectively separated from rice flour (Shih and Daigle 1997) and rice bran (Tang et al. 2003) with different carbohydrate hydrolases. But, the limited solubility of the resulting rice protein was not improved (Shih and Daigle 1997).

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Clemente (2000) reviewed the advantages of protein hydrolysates obtained by enzymatic methods in human nutrition. However, to the best of our knowledge, till now there were few works have yet been done about the preparation of safety and ideal proteins from RD.

Enzymatic hydrolysis could be influenced by many factors, including pH value, temperature, hydrolysis time and enzyme/substrate ratio. All of these could affect the enzyme activity and subsequently influence the rate of hydrolysis. In addition, the choice of protease and the degree of hydrolysis also have greatly influenced the physicochemical properties of the final hydrolysate (Liu et al. 2010). Therefore, it is necessary to investigate the protease performance during enzymatic proteolysis. As a statistical method, response surface methodology (RSM) could simultaneously explore the relationships between several variables and one or more response variables, and search for the optimum conditions for desired responses. Which has been used successfully to optimize the parameters for a number of protein hydrolysis processes (Nilsang et al. 2005).

For the purpose of developing an innovative protein preparation with added nutritive value, the aims of the current study were to select a suitable protease and optimize the proteolysis parameters with RSM for RD protein hydrolysis; meanwhile the product properties of nitrogen solubility, molecular weight distribution and amino acid composition were also identified.

Materials and methods

Materials

RD (63.50% protein, dry mater basis) were provided by Jiangxi Hengtian Co. Ltd. (Jiangxi, China). Flavourzyme, Alcalase and Neutrase were purchased from Novozymes Biotechnology Co. Ltd (Beijing, China), and Trypsin was obtained from Xueshan Enzyme Preparation Factory (Jiangsu, China). The specifications of these proteases are listed in Table 1. All of the chemicals used in this research were of analytical grade.

Methods

RD was first defatted with an equivalent volume of petroleum ether for 30 min at 50°C, and then immersed in a fivefold weight of water at 50°C for 30 min, to elute the soluble carbohydrate and improve the accessibility of the RD protein.

Selection of proteases

The defatted RD was mixed with distilled water at a ratio of 1:10 (w/v) and stirred continuously in an open vessel to obtain slurry. Sodium hydroxide (NaOH; 1 mol/L) was added to adjust the pH to a specified value for each protease (Table 1). Enzymatic hydrolysis was carried out with each individual protease at an addition of 1.0‰ weight of RD for 3 h using the hydrolysis conditions recommended by the suppliers (Table 1). Each reaction was terminated by heating the vessel in boiling water for 10 min. The slurry was then centrifuged at 5,000×g for 15 min to separate the insoluble residue, and an aliquot of the supernatant was taken out for assaying of amino nitrogen and protein in order to determine DH (Adler-Nissen 1986) and RP, respectively. The bitterness for the solutions (2%, w/w) of spray-dried hydrolysates was determined by ten panel members according to the method of Sinha et al. (2007) with a minor modification as followings. The caffeine solutions, in the concentrations of 0.050, 0.045, 0.040, 0.035, 0.030, 0.025, 0.020, 0.015, 0.010, and 0.005% (w/w), were assigned the bitterness scores of 10, 9, 8, 7, 6, 5, 4, 3, 2 and 1, respectively. The samples were judged and assigned scores relatively to these caffeine solutions. This test determined that trypsin was the optimal protease for preparing hydrolysate with RD by limited hydrolysis. All further experiments were performed with trypsin.

Optimization of experiment

To prepare the RD nutritional peptides with a relatively low degree of hydrolysis in the range of 4 to 10, a central composite uniform precision rotatable design of RSM was

Table 1 Activities and optimum hydrolysis conditions of individual proteases

Protease	EC number	Activity	Main enzymolysis site ^a	Optimum conditions	
				T (°C)	pH
Neutrase	3.4.24.28	130,000 U/g	similar, but not identical	45	7.0
Alcalase	3.4.21.62	450PU/g	broad specificity, and a preference for a large uncharged residue	55	8.5
Trypsin	3.4.21.4	4,000U/g	Arg-His, Arg-, Lys-	50	8.0
Flavourzyme	3.4.11.1	500LAPU/g	Leu-Pro, Pro-Pro	50	7.5

^a Arg-Arginine, His-Histidine, Lys-Lysine, Leu-Leucine, Pro-Proline

employed to optimize the trypsin hydrolysis conditions (enzyme to RD level, RD to water ratio, pH value, temperature, and hydrolysis time) for intensifying DH and improving RP. The five independent factors were investigated at five equidistant levels (−2, −1, 0, +1 and +2). The experimental design used for study is shown in Table 2. DH and RP for RD proteolysis were selected as the responses. According to the RSM design, 32 experimental runs (six

runs at centre point) were executed and the observations were fit to the following second order polynomial model:

$$y = \beta_0 + \sum_{j=1}^p \beta_j x_j + \sum_{j=1}^p \beta_{jj} x_j^2 + \sum_{k=1}^{p-1} \sum_{j=k+1}^p \beta_{kj} x_k x_j, \quad k < j$$

where y is the predicted response (DH or YP); β_0 is the intercept; β_j , β_{jj} and β_{kj} are coefficients estimated by the model and represent the linear, quadratic, and cross-product effects of each factor on the responses, respectively; and x_j , x_k are the code values of the independent variables.

Table 2 Experimental design and responses for the rice dregs proteolysis with trypsin

Run	Independent variables ^a					Responses ^b	
	X ₁	X ₂	X ₃	X ₄	X ₅	Y ₁	Y ₂
1	1.0	0.20	8.5	55	3.5	4.35	51.5399
2	1.0	0.20	8.5	45	2.5	7.02	60.9809
3	1.0	0.20	7.5	55	2.5	7.62	74.9844
4	1.0	0.20	7.5	45	3.5	7.34	57.7383
5	1.0	0.10	8.5	55	2.5	5.77	44.3474
6	1.0	0.10	8.5	45	3.5	7.60	73.4564
7	1.0	0.10	7.5	55	3.5	5.80	61.7890
8	1.0	0.10	7.5	45	2.5	6.05	55.0748
9	0.8	0.20	8.5	55	2.5	6.34	65.0854
10	0.8	0.20	8.5	45	3.5	7.20	60.2011
11	0.8	0.20	7.5	55	3.5	6.75	58.3660
12	0.8	0.20	7.5	45	2.5	8.69	57.8623
13	0.8	0.10	8.5	55	3.5	5.60	48.6017
14	0.8	0.10	8.5	45	2.5	7.89	60.0236
15	0.8	0.10	7.5	55	2.5	6.68	55.0671
16	0.8	0.10	7.5	45	3.5	6.27	49.2828
17	1.1	0.15	8.0	50	3.0	6.43	60.1361
18	0.7	0.15	8.0	50	3.0	7.21	50.5652
19	0.9	0.25	8.0	50	3.0	2.28	61.4613
20	0.9	0.05	8.0	50	3.0	1.50	59.2695
21	0.9	0.15	9.0	50	3.0	8.47	60.7219
22	0.9	0.15	7.0	50	3.0	9.70	57.8242
23	0.9	0.15	8.0	60	3.0	3.78	41.4239
24	0.9	0.15	8.0	40	3.0	5.57	46.1486
25	0.9	0.15	8.0	50	4.0	8.43	62.6623
26	0.9	0.15	8.0	50	2.0	6.99	59.8228
27	0.9	0.15	8.0	50	3.0	7.87	67.3511
28	0.9	0.15	8.0	50	3.0	8.24	68.3128
29	0.9	0.15	8.0	50	3.0	7.24	61.2830
30	0.9	0.15	8.0	50	3.0	6.62	63.5643
31	0.9	0.15	8.0	50	3.0	7.15	63.0048
32	0.9	0.15	8.0	50	3.0	6.92	60.8010

^a Independent variables X₁, X₂, X₃, X₄, and X₅ represent ratio of enzyme-to-rice dregs (E/R, % w/w), ratio of RD to water (R/W, w/w), pH, temperature (°C), hydrolysis time (h), respectively

^b Responses Y₁ and Y₂ represent the degree of hydrolysis (%) and recovery of protein (% w/w), respectively

Degree of hydrolysis

The degree of hydrolysis was determined using the pH-stat method (Adler-Nissen 1986) and calculated as follows:

$$\text{DH}(\%) = (\text{BN}_b) / (\text{M}_p \alpha h_{\text{tot}}) \times 100$$

where B is the amount of alkali consumed (mL), N_b is the normality of alkali, M_p is the mass of the substrate (protein in grams, % N×6.7), h_{tot} is the number of peptide bonds. For rice protein, h_{tot} = 7.40 meq/g of protein (Wang and Yao 2004). α is the calibration factor for the pH-stat, the average degree of dissociation of the amino group, calculated as follows:

$$\alpha = 10^{\text{pH}-\text{pK}} / (1 + 10^{\text{pH}-\text{pK}})$$

where pK is the average pK for $\alpha\text{-NH}_3^+$, and pH is the solution pH for initial reaction.

Protein recovery

The protein recovery was expressed as the ratio of the amount of protein in the supernatant and the amount of total protein in the raw material. The protein content was determined according to Kjeldahl method (AOAC 1998), and RP was calculated with the following formula:

$$\text{RP}(\%) = \left(\frac{\text{Protein content in the supernatant}}{\text{total protein content in the original slurry}} \right) \times 100$$

Dried protein hydrolysate preparation

Protein hydrolysis of RD was carried out with optimized conditions to ascertain the protein recovery and characteristics. After centrifugation, the supernatant was spray dried in a MDR.P-5 model spray dryer (Jiangsu, China) with a feed rate of 20 mL/min. The inlet and outlet temperatures were maintained at 195±2 and 90±2°C, respectively.

Nitrogen solubility index of the dried hydrolysate

The protein solubility of samples was evaluated by the nitrogen solubility index (Ponnampalam et al. 1987) with

minor modifications. A protein hydrolysate sample (0.4 g) was suspended in distilled water (20 mL) and the pH of the system was adjusted to the desired values (pH 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, respectively) using HCl (0.1 mol/L) or NaOH (0.1 mol/L). The suspensions were agitated by a Magnetic Stirrer (Model JB-3, Shanghai Leici Co. Ltd, China) for 30 min at 25°C and centrifuged at 7,500×g for 15 min, and then the supernatants were used to determine the amount of Kjeldahl nitrogen. The nitrogen solubility index (NSI) was calculated as follows:

$$\text{NSI}(\%) = \left(\frac{\text{Amount of nitrogen in the supernatant}}{\text{total amount of nitrogen in the sample}} \right) \times 100$$

Molecular weight distribution

The obtained hydrolysate sample (0.5 g) was dissolved with phosphate buffer (10 mL, pH 7.2) and then the molecular weight distribution was determined on a protein purification system (Amersham Biosciences-LKB) by gel filtration chromatography method. A Superdex™ 10/300 GL peptide column with a fractionation range from 100 to 7,000 Da was used, and a 25 µL sample was added to the column. The phosphate buffer (pH 7.2) containing NaCl (0.25 mol/L) was used as the elution at a flow rate of 0.5 mL/min. Wavelength of 214 nm was used for detection and quantification. Peptide standard mixtures (Amersham Biotech, GE, USA), Globin III (2,512 Da), Globin II (6,214 Da), Globin I (8,519 Da), Globin I+III (10,700 Da) supplemented with threonine (120 Da), glutathione (307 Da) and oxidized glutathione (630 Da), were used for molecular weight calibration. Threonine, glutathione and oxidized glutathione were HPLC-grades and purchased from Sigma Chemical Co. Ltd (MO, USA).

Chemical composition analysis

The chemical compositions (i.e. moisture, total crude protein, total lipid, and ash) of RD and its hydrolysate were determined according to AOAC (1998). The peptide content was assayed according to the method of Wu et al. (2003). For amino acid analysis, samples (approximately 0.5 g) were hydrolyzed for 20 h under vacuum at 110°C using HCl (10 mL, 5.8 mol/L) with 1% (w/v) phenol vapor, then pre-column derivatized with phenylisothiocyanate (10 mL) and analyzed by C₁₈ reverse phase column according to the method described by Wu and Knabe (1994). The datas for essential amino acid (EAA) were used for calculating amino acid score (Damodaran 1996) according to the following formula:

$$\text{Amino acid score} = \left(\frac{\text{EAA in test protein}}{\text{EAA in standard protein}} \right)$$

Data analysis and software

SAS V8.0e software (Statistical Analysis System Institute Inc., Cary, NC, USA) was used for the experimental design and statistical analysis of the experimental datas. One-way analysis of variance and Duncan's multiple range test were applied to determine the differences among treatments in the experiment for selecting a suitable protease, while the response surface regression procedure was used to estimate the responses of independent variables and to obtain the optimized conditions for enzymatic proteolysis under the action of the selected protease.

Results and discussion

Properties of hydrolysates with individual proteases

DH is used for monitoring the hydrolysis degree and measuring the product suitability (Adler-Nissen 1978). Adler-Nissen (1984) reported that a low bitterness can usually be ascertained by restricting DH to low values (e. g. DH 3–5%). For soy protein hydrolysate, DH of the product is 8–15%, more preferably 9.5–10.5% (Adler-Nissen 1978). Thus, the protease selection experiment was performed to screen out the suitable protease for preparing a preferable RD protein hydrolysate of low DH (4–10%).

Although many factors may affect the degree of hydrolysis and the yield of hydrolysate, the type of enzyme used in hydrolysis contributes the dominant effect on the yield and properties of the final product. In present study, the hydrolysates obtained with different proteases were significantly different in both DH and RP. The DH of hydrolysate (8.96%) obtained with trypsin was the most extensive while those for other three proteases were no more than 5.00% (4.50%, 4.09% and 4.33% for alcalase, neutrase and flavourzyme, respectively), and the RPs were both higher under the action of alcalase (58.30%) or trypsin (57.67%) than those under the action of other two proteases (53.69% and 53.87% for neutrase and flavourzyme, respectively). This may be mainly ascribed to the difference in the amount of available enzymolysis sites, which is largely dependent on the protease with itself, its specificity (Table 1) and the RD protein substrate with its specificities in amino acid composition (Table 4) and amino acid sequence. In the present study, as compared to neutrase, alcalase or flavourzyme, the extensive DH for trypsin implied that trypsin could get relatively more enzymolysis sites, which mainly consisted by Arg-, Lys-, and Arg-His (Table 1). Thus, the oligopeptides obtained through the action of

trypsin were likely to be more numerous and smaller in molecular weight than those generated with other protease treatments.

The bitterness scores of hydrolysates from neutrase, alcalase, trypsin and flavourzyme were 4.77 ± 0.25 , 5.53 ± 0.41 , 2.20 ± 0.26 and 1.97 ± 0.15 , respectively. It is obvious that the flavours from the hydrolysates treating by trypsin and flavourzyme were more pleasant than those from other hydrolysates. Matoba and Hata (1972) elucidated that hydrophobic amino acids took on a bitter taste when the α -amino and carboxyl groups were involved in peptide bond formation compared to when they occurred at the N- or C-terminus of peptides. Ishibashi et al. (1988) demonstrated that the bitter taste was weak or absent when a proline residue was located at the N-terminus of peptides but was more intense when at the center of the peptide linkages. Thus, it is clear that the specificities of different proteases endowed the hydrolysates with significantly different flavors. By comprehensively considering the properties and safety of the hydrolysate, trypsin was screened out as the optimal protease for the subsequent RD proteolysis.

Optimization of RD proteolysis with trypsin

Model fitting

The limited proteolysis condition for RD with the aid of trypsin was optimized using different variable combinations according to a central composite uniform precision rotatable design of RSM. Table 2 presents the experiment design and corresponding response data for DH and RP. The regression coefficients of the intercept, linear, quadratic, and interaction terms of model were calculated using the least squares technique and presented in Table 3 along with the t-values and the corresponding p-values, which were used as a tool to check the significance of each coefficient. The coefficients of determination (R^2) of models for DH and RP were both >0.9000 , which indicated that the models both adequately represented the real relationship between the parameters chosen. The low value of coefficient of variation (13.394 and 6.786 for DH and RP, respectively) indicated a greater reliability of the experiment.

Seen from the variance analysis for models (Table 3), the linear regressions for DH and RP were both insignificant ($p > 0.05$), the quadratic polynomial regressions of the total models for DH and RP were both striking ($p < 0.01$), and the lack fitnesses of residuals were both insignificant, which indicated that the predicted models for DH and RP both fit well. After elimination of parameters with p-values >0.25 except for the linear regression, the quadratic polynomial regression models

corresponding to DH and RP were presented as follows:

$$Y_1(\text{DH}, \%) = 7.162 - 0.227x_1 + 0.217x_2 - 0.246x_3 - 0.531x_4^* - 0.095x_5 - 0.471x_2x_3 - 0.3845x_3x_4 - 1.184x_2^{2***} + 0.614x_3^{2**} - 0.489x_4^{2*} + 0.27x_5^2$$

$$Y_2(\text{RP}, \%) = 63.251 + 1.857x_1^* + 1.813x_2^* - 0.006x_3 - 1.012x_4 - 0.280x_5 - 2.0372x_1x_3 + 1.9202x_1x_5 + 2.576x_2x_4^* - 3.105x_2x_5^{**} - 4.709x_3x_4^{***} - 1.6203x_4x_5 - 1.3739x_1^2 - 4.265x_4^{2***}$$

*Significant at 0.05 level, ** Significant at 0.01 level, ***Significant at 0.001 level; Y_1 and Y_2 are the response variables DH and RP, respectively; x_1 , x_2 , x_3 , x_4 and x_5 are coded values of the explanatory variables enzyme-to-RD ratio, RD-to-water level, pH value, temperature and hydrolysis time, respectively.

The regression models revealed that the DH and RP were relatively sensitive to variations in RD-to-water level and hydrolysis temperature, but insensitive to changes in enzyme-to-RD ratio and hydrolysis time, respectively.

Optimization of proteolysis parameters

The response surface curves of DH (Y_1) and RP (Y_2) as a function of process parameters at a constant time of 3 h were plotted to visually understand the interaction of the variables and to determine the optimum level of each variable for maximum response (Fig. 1). The nature of the saddle surface (Fig. 1a₁, a₂, b₁, b₂) signifies that the optimum condition for RD proteolysis is not unique. These graphs suggested that the maximum responses for DH and RP are both not in the stable ranges of responses surface and that the optimal conditions for DH and YP are not identical.

To search for the region of optimum proteolysis condition for RD with the aid of trypsin, ridge analysis of RSM were carried out. Considering that maximum protein recovery is always desirable rather than more intensive DH of hydrolysates in actual production, the optimal process parameters obtained with ridge analysis for RD protein enzyme reactions were: pH value, 7.6; hydrolysis temperature, 52.83°C; enzyme-to-RD ratio, 0.89:1000 (g/g); RD-to-water level of 0.22 g/mL; and hydrolysis time, 2.42 h.

Under the optimal condition, three additional experiments were conducted to validate the reproducibilities of predicted models for DH and RP. The observed values demonstrated the validity of the RSM model, since there were no significant ($p > 0.05$) differences between the observed responses (6.95 ± 0.18 , 75.81 ± 2.56) and the predicted results (6.87 ± 0.23 , 76.14 ± 2.19) for DH and RP, respectively. The strong correlation between the real and

Table 3 Analysis of variance for the model and factor tests

Item	DF	Y_1				Y_2			
		SS	R ²	F	p-value	SS	R ²	F	p-value
Model test									
Regression									
Linear	5	10.7906	0.1162	2.76	0.0748	188.0766	0.1049	2.37	0.1083
Quadratic	5	65.9028	0.7099	16.84	<0.0001	574.9432	0.3207	7.24	0.0031
Cross-product	10	7.5286	0.0811	0.96	0.5205	855.3543	0.4771	5.39	0.0051
Total Model	20	84.2219	0.9073	5.38	0.0032	1618.3741	0.9026	5.10	0.0041
Residual									
Lack of Fit	6	6.7801		3.09	0.1183	125.9999		2.16	0.2078
Pure Error	5	1.8297				48.6093			
Total Error	11	8.6098				174.6092			
Factor test									
X ₁	6	1.8245	0.3041	0.39	0.8715	296.5356	49.4226	3.11	0.0491
X ₂	6	46.5678	7.7613	9.92	0.0007	376.6663	52.7772	3.95	0.0235
X ₃	6	18.4776	3.0796	3.93	0.0239	465.3666	77.5611	4.89	0.0114
X ₄	6	16.6834	2.7806	3.55	0.0331	1073.9702	178.9950	11.28	0.0004
X ₅	6	3.7136	0.6189	0.79	0.5956	280.4614	46.7436	2.94	0.0576

Fig. 1 Response surface graphs for DH (Y_1) and RP (Y_2) as a function of process parameters during hydrolysis of RD with trypsin (hydrolysis time at the center level; 3 h). Independent variables X_1 , X_2 , X_3 and X_4 , represent ratio of enzyme-to-rice dregs (E/R, % w/w), ratio of RD to water (R/W, w/w), hydrolysis temperature (°C), respectively; T represents hydrolysis temperature (°C). (**a**₁. Fixed levels: pH=8, $T=50^\circ\text{C}$; **b**₁. Fixed levels: E/R=0.9, R/W=0.15; **a**₂. Fixed levels: pH=8, $T=50^\circ\text{C}$; **b**₂. Fixed levels: E/R=0.9, R/W=0.15)

the predicted responses confirmed that the response models for DH and RP were adequate to reflect the expected optimization. It signified that predicted values of the models corresponded well with the experimental results.

Protein solubility with various pH

The solubility is a good index of potential applications of proteins, because many functional performances of proteins depend upon their capacity to go initially into solution. Chi et al. (2003) demonstrated that the protein solubility is largely dependent on the properties of the solution environment as well as the relative intrinsic thermodynamic

stability of the native state. As seen in Fig. 2a, the nitrogen solubilities of RD and its hydrolysates were pH independent over the range tested. The pH-solubility profiles showed that RD hydrolysate had a good solubility above 75% over a wide pH range, with a minimum solubility at the isoelectric pH, where the electrostatic repulsion and ionic hydration were minimal and hydrophobic interaction between surface nonpolar patches was maximal. The solubility profile exhibits an increase in the pH solubility of RD protein hydrolysate over that of the RD protein. This type of behavior could be due to the reduction of its secondary structure and the release of smaller polypeptide units from the protein during the enzymatic proteolysis (Radha et al. 2008). It is conceivable that smaller, more hydrophilic and more solvated polypeptide units are produced as a consequence of enzymatic hydrolysis (Damodaran 1996). This is an important feature, which could increase the use of RD hydrolysates in many food and nonfood applications.

Molecular weight distribution of the prepared hydrolysate

Figure 2b shows the gel filtration chromatographic profile of RD hydrolysate obtained under the optimum process condition with the aid of trypsin. Molecular weight distribution results demonstrated that 18.7% of the peptides were lower than 120 Da, 52.2% in the range of 120 to 1,000 Da, 18.4% in the range of 1,000 to 3,000 Da and 5.6% above 10,000 Da. This suggested that RD proteins were broken into oligopeptides under the cleavage reaction of the selected trypsin protease. Cleavage of peptide linkages is usually tending to expose some hydrophobic regions originally buried within the protein molecule to the aqueous phase, which is associated with the important structural rearrangement of protein molecule. Research has revealed that the partial hydrolysis (Sekul et al. 1978) and enzymatic modification (Bhagya and Srinivasan 1989) have effects on the molecular weight distribution, and surface hydrophobicity have an important influence on the emulsifying capacity as well as solubility and foaming capacity of the final hydrolysate. Therefore, the products of enzymatic hydrolysate are likely to possess some innovative properties as compared to RD material.

Chemical composition

Approximate composition

The spray dried RD hydrolysate was a milky white powder. As can be seen from Table 4, RD had a protein content of 63.5% with a higher fat content close to 9% and a higher carbohydrate content of >11%. In the present study, most of the lipid was extracted by petroleum ether and most of the

Fig. 2 Characterizations of water-solubility and gel filtration chromatography for the rice dregs hydrolysate obtained under the optimum process condition with the aid of trypsin. **a** The pH-solubility profiles of RD hydrolysis and RD protein in distilled water at 25°C; **b** The chromatographic elution profile of rice dregs hydrolysate with a Superdex™ peptide 10/300 GL column

Table 4 The amino acid composition of RD hydrolysate (g/100 g crude protein, dry basis) and its amino acid scores calculated basing on FAO and NRC reference proteins

Amino acid	RD	RD hydrolysate	Reference protein 1 ^a	Reference protein 2 ^b	Amino acid scores for hydrolysate	
					RP-1 ^c	RP-2 ^d
Essential amino acids						
Leucine	5.51	5.15	1.90	5.00	2.71	1.26
Histidine	1.54	1.80	1.60	1.67	1.13	1.25
Phenylalanine +Tyrosine	7.35	9.63	1.90	4.83	5.07	2.31
Methionine +Cystine	3.24	5.71	1.70	3.00	3.36	2.15
Isoleucine	2.41	5.15	1.30	2.83	3.96	2.06
Valine	3.75	4.91	1.30	3.56	3.78	1.62
Threonine	2.11	3.04	0.90	3.39	3.38	0.98
Lysine	2.01	2.79	1.60	5.28	1.74	0.61
Non-essential amino acids						
Glutamate	13.91	14.51				
Aspartic acid	5.79	7.88				
Arginine	5.00	7.28				
Serine	3.58	4.43				
Glycine	2.87	3.71				
Alanine	3.70	4.13				
Approximate composition						
Crude protein	63.50	81.42				
Peptide	1.91	74.27				
Fat	8.88	1.10				
Ash	7.89	5.05				
Carbohydrate	11.20	2.76				

^a Reference protein 1: Essential amino acid requirement for adults according to FAO/WHO/UNU(1985), (mg/g crude protein, dry basis)

^b Reference protein 2: Essential amino acid requirements for pig of 20–50 kg body weight according to NRC(1998), (mg/g crude protein, dry basis)

^c RP-1: amino acid scores calculated with FAO/WHO/UNU reference protein as the base

^d RP-2: amino acid scores calculated with amino acid requirements as per NRC (1998)

carbohydrate was eluted by the warm water, to give final contents of fat and carbohydrate (1.10% and 2.76%, respectively) in the product; the available peptide content in the hydrolysate was pronouncedly increased to 74.27%. It appears that the pretreatment of RD could significantly improve the protein level in the substrate and might subsequently reduce the negative effects of fat, which has a contribution to decrease the protein solubility, the protein thiols modification (Boatright and Hettiarachchy 1995a, b) and the final product instability (Nilsang et al. 2005).

Amino acid composition

The result of amino acid analysis for RD and dried hydrolysate prepared using the optimal condition is presented in Table 4. As the result revealed, there was a great increase for each amino acid (except for leucine) in the dried hydrolysate comparing with the original material. This could mainly be ascribed to the purifying effect of pretreatment adopted,

though the modification of the amino acid loss during the centrifugation after hydrolysis should not be ignored.

The quality of proteins and its capacity to fulfill the needs of organism with respect to EAAs determine the nutritive quality of any ingredient. In present study, the amino acid score was applied to assess the protein quality of RD hydrolysate, based on the reference protein of FAO/WHO/UNU (1985) and amino acid requirements of pigs as listed in NRC (1998). The amino acid scores calculated (Table 4) indicated that all the amino acids in dried hydrolysate are in sufficient or excess quantity required for an adult, and also as required for pigs, except for lysine. This means that lysine is the limiting amino acid in dried RD hydrolysate as for pig. Which is a common nutritional defect of plant proteins in terms of the needs of organisms, but could be improved by supplementation with commercial amino acids. On the other hand, the abundance of glutamate and arginine in the dried hydrolysate suggest that this may be its strength in nutrition, especially for the infants and neonates. Because glutamate is an obligatory

amino acid for intestinal mucosal mass and integrity (Wu 1998; Reeds and Burrin 2000) and defenses of mucosa against toxic and peroxidative damage (Reeds et al. 1997). A relatively high level of arginine is necessary for maximal growth of the neonate (Wu et al. 2004), acting to induce secretion of growth hormone and insulin-like growth factor I (Le Floc'h et al. 2004), stimulating muscle protein synthesis, and promoting weight gain in neonatal pigs (Yao et al. 2008).

Furthermore, amino acids in the presence of peptide are more efficiently utilized than free amino acids and whole protein. Peptides are also less hypertonic than the free amino acid mixtures, which could enable the improved absorption of protein components and eliminate osmotic problems (Clemente 2000). Hence, in spite of minor deficiencies in certain necessary amino acids, the dried protein hydrolysate does not lose its nutritional value and has the potential to be an important protein ingredient in the milk replacers and in the balanced diets for patients with enteric illnesses.

Conclusions

In this study, a new protein hydrolysate was obtained from RD using trypsin as the protease with more than 75% of RP and less than 9% of DH, respectively. The DH and RP were significantly influenced by RD with respect to concentration, pH, and hydrolysis temperature. The optimal parameters obtained were: pH value of 7.6, hydrolysis temperature of 52.83°C, enzyme-to-RD ratio of 0.89:1000(g/g) and RD-to-water ratio of 0.22:1(g/ml), hydrolysis time of 2.42 h. The final hydrolysate shows a good solubility in the range of pH 2–11, a low molecular weight distribution, and is also abundant in EAA, and hence is potentially an excellent protein ingredient for good quality in the food and feed industries.

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References

- Adler-Nissen J (1978) Hydrolysis of soy protein. US Patent 4 100 024
- Adler-Nissen J (1984) Control of the proteolytic reaction and of the level of bitterness in protein hydrolysis processes. *J Chem Technol Biotechnol* 34(3):215–222
- Adler-Nissen J (1986) *Enzymic hydrolysis of food proteins*. Elsevier Applied Science Publishers Ltd., New York, pp 132–142
- AOAC (1998) *Official methods of analysis*, 16th edn. Association of official analytical chemists, Washington
- Bhagya S, Srinivasan KS (1989) Effect of different methods of drying on functional properties of enzyme treated ground flour. *J Food Sci Technol* 22:329–333
- Boatright WL, Hettiarachchy NS (1995a) Effect of lipids on soy protein isolate solubility. *J Am Oil Chem Soc* 72:1439–1444
- Boatright WL, Hettiarachchy NS (1995b) Lipid components that reduce protein solubility of soy protein isolates. *J Am Oil Chem Soc* 72:1445–1451
- Chi EY, Krishnan S, Randolph TW, Carpenter JF (2003) Physical stability of proteins in aqueous solution: mechanism and driving forces in nonnative protein aggregation. *Pharm Res* 20:1325–1336
- Clemente A (2000) Enzymatic protein hydrolysates in human nutrition. *Trends Food Sci Technol* 11:254–262
- Damodaran S (1996) In: Fennema OR (ed) *Food chemistry*, 3rd edn. Marcel Dekker, Inc, New York, p 400
- FAO/WHO/UNU (1985) *Energy and protein requirements*, report of a joint FAO/WHO/UNU Expert Consultation. Series No.724. World Health Organization Technical Rep. Geneva, p 121
- Haard NF (2001) Enzymatic modification of protein in food system. In: *Chemical and functional properties of food proteins*. Technomic Publishing Co. Inc, New York, pp 155–190
- Helm RM, Burks AW (1996) Hypoallergenicity of rice protein. *Cereal Foods World* 41:839–843
- Ishibashi N, Kubo T, Chino M, Fukui S, Shinoda I, Kikuchi E, Okai H, Fukui S (1988) Taste of proline—containing peptides. *Agric Biol Chem* 52:95–98
- Jiamyangyuen S, Srijesdaruk V, Harper WJ (2005) Extraction of rice bran protein concentrate and its application in bread. *Songklanakarin J Sci Technol* 27:55–64
- Le Floc'h N, Melchior D, Obled C (2004) Modifications of protein and amino acid metabolism during inflammation and immune system activation. *Livest Prod Sci* 87:37–45
- Liu Q, Kong B, Xiong Y, Xia X (2010) Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. *Food Chem* 118:403–410
- Matoba T, Hata T (1972) Relationship between bitterness of peptides and their chemical structures. *Agric Biol Chem* 36:1423–1431
- Nilsang S, Lertsiri S, Suphantharika M, Assavanig A (2005) Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. *J Food Eng* 70:571–578
- NRC (1998) *Nutrient requirements of swine: 10th revised edition*, Subcommittee on Swine Nutrition, Committee on Animal Nutrition, Board on Agriculture (BOA), p 18
- Ponnampalam R, Goulet G, Amiot J, Brisson GJ (1987) Some functional and nutritional properties of oat flours as affected by proteolysis. *J Agric Food Chem* 35:279–285
- Radha C, Ramesh KP, Prakash V (2008) Preparation and characterization of a protein hydrolysate from an oilseed flour mixture. *Food Chem* 106:1166–1174
- Reeds PJ, Burrin DG (2000) The gut and amino acid homeostasis. *Nutr* 16:666–668
- Reeds PJ, Burrin DG, Stoll B, Jahoor F, Wykes L, Henry J, Frazer ME (1997) Enteral glutamate is the preferential source for mucosal glutathione synthesis in fed piglets. *Am J Physiol* 273:E408–E415
- Sekul AA, Vinnett CH, Ory RL (1978) Some functional properties of peanut proteins partially hydrolyzed with papain. *J Agric Food Chem* 26:855–858
- Shih F, Daigle K (1997) Use of enzymes for the separation of protein from rice flour. *Cereal Chem* 74:437–441
- Sinha R, Radha C, Prakash J, Kaul P (2007) Whey protein hydrolysate: functional properties, nutritional quality and utilization in beverage formulation. *Food Chem* 101:1484–1491

- Tang SH, Hettiarachchy NS, Eswaranandam S (2003) Protein extraction from heat-stabilized defatted rice bran: II. The role of amylase, cellulase, and viscozyme. *J Food Sci* 68:471–475
- Wang Z, Yao H (2004) Determination of conversion factor of percent nitrogen to percent protein in rice protein. *Food Sci* 25:66–67
- Wu G (1998) Intestinal mucosal amino acid catabolism. *J Nutr* 128:1249–1252
- Wu G, Knabe DA (1994) Free and protein-bound amino acids in sow's colostrum and milk. *J Nutr* 124:415–424
- Wu H, Chen H, Shiau C (2003) Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Res Int* 36:949–957
- Wu G, Knabe DA, Kim SW (2004) Arginine nutrition in neonatal pigs. *J Nutr* 134:S2783–S2790
- Yao K, Yin YL, Chu W, Liu Z, Deng D, Li T, Huang R, Zhang J, Tan B, Wang W, Wu G (2008) Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. *J Nutr* 138:867–872