ISSN 1330-9862
(FTB-2270)

original scientific paper

Characterization and Preparation of Broken Rice Proteins Modified by Proteases

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Received: April 8, 2009
Accepted: September 30, 2009

Summary

Broken rice is an underutilized by-product of milling. Proteins prepared from broken rice by treatments with alkaline protease and papain have been characterized with regard to nutritional and functional properties. The protein content and the protein recovery were 56.45 and 75.45 % for alkaline protease treatment, and 65.45 and 46.32 % for papain treatment, respectively. Protease treatment increased the lysine and valine content, leading to a more balanced amino acid profile. Broken rice proteins had high emulsifying capacity, 58.3–71.6 % at neutral pH, and adequate water holding capacity, ranging from 1.96 to 2.93 g/g of proteins. At pH=7.0, the broken rice protein had the highest water holding capacity and the best interfacial activities (emulsifying capacity, emulsifying stability, foaming capacity and foaming stability), which may be the result of the higher solubility at pH=7.0. The interfacial activities increased with the increase in the mass fraction of broken rice proteins. The proteins prepared by the papain treatment had higher water holding capacity ($p>0.05$), emulsifying capacity ($p<0.05$) and foaming capacity ($p>0.05$) than alkaline protease treatment at the same pH or mass fraction. To test the fortification of food products with broken rice proteins, pork sausages containing the proteins were prepared. Higher yield of the sausages was obtained with the increased content of broken rice proteins, in the range of 2.0–9.0 %. The results indicate that broken rice proteins have potential to be used as the protein fortification ingredient for food products.

Key words: broken rice protein, nutritional properties, functional properties

Introduction

Rice is one of the important food crops in the world, and rice protein is nutritious, hypoallergenic (*1*), and suitable for application in infant food formulations and other nutraceutical foods (*2*,*3*). Broken rice, one of the major by-products generated during milling, contains about 8 % protein. Rice protein is commonly extracted using alkali solubilization (*4*) or protease modification (*5*). Alkali solubilization may cause the loss of most of the hydrophilic proteins when they are dissolved in alkali and precipitated in acid (*6*), and enzymatic hydrolysis of proteins could produce fragmental and bitter peptides, but the latter could be debittered with several treatments, which makes enzymatic modification more preferable.

The development of new food items calls for the most precise information about the characteristics and functional properties of the proteins. Chandi and Sogi (*4*) concluded that functional properties of rice bran protein concentrates were approximately equivalent to those of casein. Hamada (*5*) showed that solubility and emulsification properties of rice bran proteins were better when prepared with the protease blends. The distribution of protein fractions in oat bran differs from that of oat flour (*7*), and prolamin and glutelin are stored into dif-

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ferent sites in rice seed (*8*), which may lead to different characteristics and functional properties of broken rice proteins from those reported for rice bran proteins. So far, functional properties of broken rice protein have not been reported. The objective of the present investigation is to prepare broken rice proteins with proteases and characterize their functional properties. Also, pork sausages containing broken rice proteins have been prepared to test the application of proteins as fortifiers of food products.

Materials and Methods

Materials

The broken rice used in the experiment was supplied by Zhengzhou Fangxin Rice Co., Ltd. (Zhengzhou, PR China). Alkaline protease and papain were purchased from Nanning Pangbo Biological Engineering Co., Ltd. (Nanning, PR China). The tested enzyme activity of alkaline proteinase was 52 792.65 U/g and that of papain was 39 775.03 U/g.

The sausages were prepared according to Yang *et al.* (*9*) with small modifications. The pork loins (pH=(5.7±0.1)) and fat were purchased from a local supermarket, ground through a 3-mm plate, and then mixed thoroughly with broken rice protein, ice water and salt one by one using a mixer (Yingbo Machinery Company, Xiamen, PR China) for 5 min at 4 °C to prepare the mixture. The formulation of each batch was the following (in %): pork of the hind leg 54–63, fat 25, ice water 10, sodium chloride 2, sodium phosphate 0.3, sodium nitrite 0.002, and broken rice protein 0–9.

After mixing, the mixtures were stuffed into synthetic cellulose tubes (diameter of approx. 30 mm) using a stuffer. The sausages were left for 24 h at 4 °C to allow for the ingredients to equilibrate. The sausage samples were cooked in a water bath at 80 °C for 30 min and then cooled to 12 °C. The addition of different amounts of protein (2, 4, 6, 8 and 9 %) was tested. Control sausages were prepared without the addition of broken rice protein. All measurements were carried out in triplicate. The cooking yield of sausages (Y) was defined as follows:

$$Y=(m_3-m_1)/(m_2-m_1)\times 100 \qquad /1/$$

where m_1 is the mass of synthetic cellulose tube, m_2 is the total mass of the sausage mixtures in the synthetic cellulose tube before cooking in a water bath, and m_3 is the total mass of the cooked sausage in the synthetic cellulose tube.

Protein hydrolysis

All reactions were performed in a thermostatically controlled water bath with constant agitation (200×*g*), and the characteristics taken into consideration are shown in Table 1. A mass of 50 g of broken rice was ground to rice flour to pass through a BS50 mesh screen, and distilled water was added to the broken rice flour in a ratio of 10:1 (by volume per mass) for alkaline protease, or 20:1 (by volume per mass) for papain. The preparation of proteins with alkaline protease was carried out at pH=10.0 and 45 °C, the mass fraction of alkaline protease was 18 % (on the basis of rice flour mass) and the final activity of the enzyme in the reaction system was 950.27 U/mL. The preparation of proteins with papain was carried out at pH=7.0 and 50 °C, the mass fraction of papain was 12 % (on the basis of rice flour mass) and the final activity of papain in the reaction system was 238.65 U/mL. During the reaction, the desirable pH value of the solution was kept by the addition of 0.2 mol/L NaOH. The reaction was terminated by putting the reaction vessel into the water bath (100 °C for 10 min for alkaline protease; 100 °C for 15 min for papain) with stirring to inactivate the proteases. After the enzyme liquid cooled to room temperature (25±3) °C, the supernatant was separated through centrifugation at 4000×*g* for 8 min at room temperature (25±3) °C, the centrifugations were performed at the same room temperature throughout the text if not stated otherwise. The supernatant was collected and lyophilized. The percent protein recovery was calculated as the ratio of protein in the supernatant to the total protein of broken rice.

Composition analysis and degree of hydrolysis of protein hydrolysates

The samples were analyzed for protein, ash, fat, starch and moisture content according to AOAC methods (*10*). Their contents were expressed as the percentage on dry mass basis. Degree of hydrolysis (DH) was calculated by measuring the amount of consumed alkali (*11*).

Amino acid analysis was performed as described by Tang *et al.* (*3*) with slight modifications. Protein samples (2 mg) were hydrolyzed with 6 mol/L HCl at 110 °C for 24 h under inert nitrogen atmosphere and derived using diethyl (ethoxymethylene)malonate. Amino acids were analyzed by reversed phase high-performance liquid chromatography (RP-HPLC) using a Zorbax 80A C_{18} column (180×4.6 mm i.d.) in an Agilent 1100 assembly system (Agilent Technologies, Palo Alto, CA, USA).

Table 1. Characteristics of broken rice protein samples

Sample	Sample characteristics						
	Temperature/ °C	pH	Time/min	E/S	*w*(enzyme)/%	DH/%	Solubility at pH=7/%
Protein modified by alkaline protease (PA)	45	10.0	120	10:1	18	8.38±0.11	89.56
Protein modified by papain (PP)	50	7.0	120	20:1	12	9.12±0.24	81.63

Difference between DH of PA and PP was not significant ($p>0.05$); values are means of duplicate determinations±S.D.

Determination of water holding capacity

Water holding capacity of the broken rice proteins was determined by the method of Chandi and Sogi (*4*) with slight modifications. Water (20 mL) was added to 1 g of dry protein sample, stirred evenly and left for 20 min for water to be fully absorbed. The residue was weighed after centrifugation at 1000×*g* for 5 min. The water holding capacity was defined as the gain in mass per gram of protein.

Determination of emulsifying properties

Emulsifying activity and stability were determined by the method of Yasumatsu *et al.* (*12*) with slight modifications. Proteins were dissolved in 50 mL of distilled water to prepare the samples of various mass fractions (1.0, 3.0, 5.0 and 7.0 %) and various pH (5.0, 7.0 and 9.0) with 0.1 mol/L NaOH solution. A volume of 50 mL of protein solution was homogenised with 50 mL of peanut oil at 10 000×*g* for 2 min, and divided into two equal parts. One part was centrifuged at 1500×*g* for 10 min. The heights of the emulsified layer and that of the total contents were measured. The emulsifying capacity (EC) was calculated as follows:

$$EC = h_1/h_0 \times 100 \qquad /2/$$

where h_1 is the height of emulsified layer in the tube and h_0 is the height of the total content in the tube.

The other part was heated in the water bath at 50 °C for 30 min and then centrifuged to determine the emulsion stability at the intervals of 20, 30, 60 and 90 min. Emulsification stability was expressed as the percentage of emulsifying activity that remained at the same intervals after heating (*13*).

Determination of foaming properties

Foaming properties of the broken rice protein were determined by the method of Agboola *et al.* (*14*) with slight modifications. The protein samples were dissolved in 100 mL of distilled water to the various mass fractions (1.0, 3.0, 5.0 and 7.0 %) adjusted to various pH (5.0, 7.0 and 9.0) with NaOH solution (0.1 mol/L), and homogenized at 10 000×*g* for exactly 2 min. The volume of the foam was measured just after homogenization. Foaming capacity was calculated as the ratio of the volume of foam to the original volume (100 mL) expressed in percentage. Foam stability was expressed as the ratio of foam volume remaining after 20, 30, 60 and 90 min (in percentage).

Statistical analysis

Experiments on the functional properties of broken rice proteins were carried out in triplicate. Analysis of variance (ANOVA) was performed using an SPSS package (SPSS v. 10.0 for Windows, SPSS Inc., Chicago, IL, USA).

Results and Discussion

Chemical composition of broken rice flour and protein preparation

After the enzymatic hydrolysis, the content of protein modified by alkaline protease (PA) and protein modified by papain (PP) increased to 56.45 and 65.45 %, respectively, compared with that of the broken rice flour (8.26 %, Table 2), and higher than the protein content of a rice bran enzymatic extract (38.1 %) reported by Parrado *et al.* (*15*). The percentage of the recovery of protein modified with alkaline protease was higher than that modified with papain, although both alkaline protease and papain are endoproteases, and have a similar DH ($p>0.05$). The data on protein recovery differ from those reported by Hamada (*5*), who obtained protein recovery of 81.4 % for Alcalase® 2.4 L and 87.6 % for Flavourzyme®, both with similar percentages of DH. The disagreement could be due to the higher pH (pH=10.0) used for the alkaline protease treatment in the experiment and the different source of proteases. Their high protein content suggests that PA and PP have a potential use as the protein fortification ingredients in a variety of food products for protein-deficient people in developing countries.

Amino acid composition

The essential amino acid composition of broken rice flour, PA and PP is shown in Table 3. There was a significant increase in the lysine content of PA and PP, being 4.7 and 4.6 g per 100 g of protein, respectively, compared to 4.0 g per 100 g of protein in the broken rice flour. In addition, the contents of methionine, cystine, valine and leucine increased. These changes in the amino acid composition are probably the result of several factors: (*i*) increased level of lysine, methionine, cystine, valine and leucine in the proteins modified with proteases compared to total proteins in broken rice flour, (*ii*) partial loss of some proteins during extraction procedure (protein recovery is 46.32 and 75.45 %), and possibly (*iii*) amino acid composition of enzymes (alkaline protease and papain) used in protein hydrolysis. The content of lysine, glutamic acid (with glutamine), histine and isoleucine in broken rice proteins was lower, but the con-

Table 2. Chemical composition of different broken rice products

Sample	*w*(protein)/%	*w*(ash)/%	*w*(moisture)/%	*w*(carbohydrate)/%	*w*(fat)/%	*w*(protein recovery)/%
Broken rice flour	$(8.26\pm0.02)^a$	$(0.60\pm0.02)^a$	$(14.37\pm0.26)^a$	64.38±0.56	0.39±0.02	–
PA	$(56.45\pm0.25)^b$	$(1.23\pm0.05)^b$	$(7.23\pm0.07)^b$	–	–	$(75.45\pm0.38)^a$
PP	$(65.45\pm0.22)^b$	$(1.11\pm0.06)^b$	$(6.56\pm0.06)^b$	–	–	$(46.32\pm0.34)^b$

Values superscripted with different letters (a, b) in the same columns differ significantly ($p<0.05$); values are means of duplicate determinations±S.D.

Table 3. Amino acid composition (g per 100 g of protein) of different broken rice products

Essential amino acid	*w*(PA) %	*w*(PP) %	*w*(broken rice flour) %	*w*(FAO scoring pattern)* %
Lysine	4.7	4.6	4.0	5.8
Glutamic acid+ glutamine	3.2	2.9	3.3	1.9
Histidine	2.0	1.5	1.7	3.4
Methionine+ cystine	5.0	3.9	3.7	2.5
Valine	8.1	6.4	5.8	3.5
Leucine	8.7	8.8	8.2	6.6
Isoleucine	3.1	4.0	4.1	2.8
Phenylalanine+ tyrosine	8.4	7.8	10.3	6.3

*Data taken from FAO/WHO/UNU (*16*)

Table 4. Water holding capacity of broken rice proteins under different pH (at 18 °C) and temperatures (at pH=8.0)

		Water holding capacity of broken rice proteins g/g		Water holding capacity of rice bran protein concentrates* g/g
		PA	PP	
pH	4.0	$(1.96\pm0.47)^{ax}$	$(2.03\pm0.16)^{ax}$	3.87–5.60
	5.0	$(2.08\pm0.26)^{ax}$	$(2.23\pm0.11)^{axy}$	
	6.0	$(2.13\pm0.03)^{ax}$	$(2.34\pm0.90)^{by}$	
	7.0	$(2.25\pm0.08)^{ay}$	$(2.45\pm0.18)^{by}$	
t/°C	25	$(2.86\pm0.32)^{ay}$	$(2.75\pm0.30)^{ay}$	
	35	$(2.93\pm0.36)^{ay}$	$(2.89\pm0.28)^{ay}$	
	50	$(2.42\pm0.38)^{ax}$	$(2.45\pm0.16)^{ax}$	
	60	$(2.25\pm0.15)^{ax}$	$(2.32\pm0.31)^{ax}$	

Values superscripted with different letters in rows (a, b) and columns (x, y, z) are significantly different at p=0.05; values are means of triplicate determinations±S.D.; *data taken from Chandi and Sogi (*4*)

tent of valine, leucine and phenylalanine (with tyrosine) was similar to that in rice bran proteins reported by Tang *et al.* (*3*). The results indicate that the PA and PP had more balanced amino acid profiles.

Water holding capacity of broken rice protein

Water holding capacity of protein reflects the degree of hydration, it is closely related to 'keeping shape' in the food storage, and is affected by pH, temperature and ionic strength (*17*). The results are presented in Table 4.

The rice protein hydrolysates had the water holding capacity ranging from 1.96 to 2.93 g/g proteins. Water holding capacity of PP was higher than that of PA at the same pH, which may be due to their different mode of action; while the water holding capacity of broken rice protein reached the lowest point at pH=4.0, and then increased with the increase in pH. The highest water holding capacities of PA and PP were at 35 °C, and then decreased with the increase of temperature at pH=8.0. The differences between them were not significant (p>0.05). Water holding capacities of PA and PP were lower than those of rice bran protein concentrates (*4*), Australian lentil cultivars (*6*) and those of protein concentrates from pressed cakes of Chilean hazelnut (*18*) but higher than those of the defatted meal and the protein isolates of Ethiopian mustard (*19*).

Emulsifying properties of broken rice proteins

Emulsifying properties of broken rice proteins were studied under different pH and mass fractions, and the results are shown in Table 5. Broken rice proteins had the highest emulsifying capacity at neutral pH, 58.3–71.6 %, and the lowest at pH=5.0, which could be attributed to the decrease in solubility. This is similar to the change of the emulsifying properties of rice bran protein concentrate (*4*).

Emulsifying capacity and stability of broken rice proteins was mass fraction-dependent (p<0.05), and increased as the mass fraction of broken rice proteins increased at pH=7.0 and room temperature (25±3) °C. Proteins are surface-active and facilitate an oil-in-water emulsion because of their hydrophilic and hydrophobic side chains

Table 5. Emulsifying capacity and emulsifying stability of broken rice proteins under different pH (*w*=5 % at 25 °C) and mass fractions (pH=7.0 at 25 °C)

Parameters		PA/%				PP/%			
		Emulsifying capacity	Emulsifying stability			Emulsifying capacity	Emulsifying stability		
Time/min		0	20	60	90	0	20	60	90
pH	5.0	$(57.0\pm3.1)^{x}$	$(62.2\pm1.6)^{cx}$	$(41.5\pm2.2)^{bx}$	$(32.2\pm2.1)^{ax}$	$(67.1\pm3.5)^{y}$	$(58.3\pm2.4)^{cx}$	$(36.5\pm1.8)^{bx}$	$(29.5\pm1.6)^{ax}$
	7.0	$(58.3\pm3.4)^{x}$	$(65.6\pm2.2)^{bx}$	$(51.4\pm2.1)^{aby}$	$(45.3\pm2.6)^{ay}$	$(71.6\pm3.4)^{y}$	$(65.5\pm2.1)^{cx}$	$(43.6\pm2.2)^{by}$	$(32.2\pm1.6)^{ax}$
	9.0	$(51.4\pm3.6)^{x}$	$(62.3\pm1.9)^{cx}$	$(47.1\pm2.2)^{abxy}$	$(42.7\pm1.6)^{ay}$	$(54.3\pm3.4)^{x}$	$(56.7\pm2.3)^{cx}$	$(41.9\pm2.1)^{by}$	$(26.6\pm1.2)^{ax}$
w(protein)/%	1.0	$(32.2\pm2.6)^{x}$	$(23.3\pm1.1)^{cx}$	$(12.3\pm1.6)^{bx}$	$(8.0\pm0.9)^{ax}$	$(58.2\pm2.5)^{x}$	$(35.3\pm1.6)^{cx}$	$(18.3\pm1.5)^{bx}$	$(5.0\pm0.1)^{ax}$
	3.0	$(45.2\pm2.5)^{y}$	$(33.6\pm1.3)^{cy}$	$(15.5\pm1.0)^{bx}$	$(10.2\pm0.5)^{ax}$	$(65.2\pm2.4)^{y}$	$(43.6\pm1.5)^{bxy}$	$(19.5\pm1.1)^{ax}$	$(15.6\pm0.6)^{ay}$
	5.0	$(56.6\pm2.5)^{z}$	$(42.4\pm2.2)^{cyz}$	$(21.4\pm1.9)^{bxy}$	$(13.2\pm0.8)^{axy}$	$(70.5\pm2.6)^{yz}$	$(43.9\pm1.2)^{bxy}$	$(25.8\pm1.5)^{ay}$	$(21.1\pm0.9)^{ayz}$
	7.0	$(63.4\pm1.6)^{z}$	$(55.1\pm3.1)^{cz}$	$(27.0\pm2.0)^{by}$	$(18.6\pm1.2)^{ay}$	$(74.1\pm1.6)^{z}$	$(46.5\pm2.2)^{by}$	$(23.8\pm1.5)^{axy}$	$(22.3\pm1.0)^{az}$

Values superscripted with different letters in rows (a, b, c) and columns (x, y, z) are significantly different (p<0.05); values are means of triplicate determinations±S.D.

and their charge. The increase in the protein mass fraction in the solution resulted in an increase in the rate of diffusion of protein (*20*), a higher protein mass fraction at the interface, and thus the higher emulsifying capacity. Thiansilakul *et al.* (*21*) observed the opposite trend. The divergence may be caused by the different source and degree of hydrolysis of the materials, and also by the method used in the studies. PP showed higher emulsifying activity than PA. PA contained more non-protein constituents, which may impair its emulsifying capacity. The emulsifying capacity of broken rice proteins was higher than that of rice bran protein concentrate (*4*), which indicates that the broken rice proteins are more suitable for application in hypoallergenic sausages, soups and salad dressings.

Foaming properties of broken rice proteins

The foaming capacity and foam stability are related to pH (*4*), mass fraction and ionic strength (*22*). Foaming capacity and stability of broken rice proteins under different pH and mass fractions are shown in Table 6.

Both PA and PP showed the highest foaming capacity (65.6 and 73.3 %) and foam stability at pH=7.0, and the lowest at pH=5.0. The foaming capacity and foaming stability of broken rice proteins are mass fraction-dependent (at pH=5.0 and room temperature, both siginificant at $p<0.05$), and increased with the increase in the mass fraction of 1.0–7.0 %. For PA, the foaming capacity and stability reached 65.1 and 55.6 %, respectively (at 20 min) at 7.0 % solid mass fraction, and 38.3 and 23.2 % (at 20 min) at 1.0 % solid mass fraction. These results are in agreement with those obtained for the foaming properties of pigeon pea protein concentrate (*23*), chickpea flour (*24*) and protein isolates from different Indian chickpea cultivars (*25*). The foam volume decreased with the lapse of time. A similar trend has been reported for mucuna bean protein concentrates (*26*), Ethiopian mustard protein isolates (*19*) and protein isolates from different Indian chickpea cultivars (*25*).

At the same pH or the mass fraction, PP had higher foaming capacity and foaming stability than PA, which was not significant ($p>0.05$). This difference may reflect differences in protein molecular size and conformation because of the different mode of action of the proteases.

Aplication of broken rice proteins in sausages

The pork muscle contains about 75 % water, but loses water-holding capacity after heating, thus the prepared product has low yield and hard texture. The key to the production of pork sausage is in retaining water and fat (*26*). The results in Fig. 1 showed that the cooking yield of sausages increased by increasing the protein content. The cooking yield of the sasauge was significantly ($p<0.05$) higher with 9.0 % PP than that without PP (66.23 %), while with 0, 2.0 and 9.0 % PA, the yields were 63.14, 72.32 and 78.32 %, respectively. Higher levels of broken rice protein produced higher yields, indicating that the addition of broken rice protein is useful for retaining water in the product during cooking. The water holding capacity of sausages was greater when the level of added broken rice protein increased. Similarly, Dzudie *et al.* (*27*) reported that the water holding capacity increased and cooking loss decreased with the

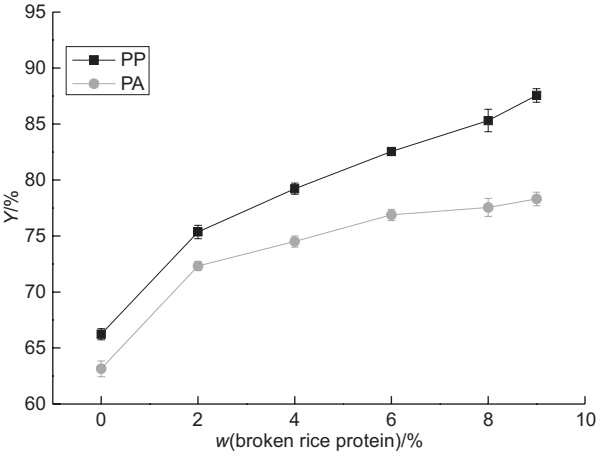

Fig. 1. The cooking yield of sausage products with different mass fractions of broken rice protein

Table 6. Foaming capacity and foaming stability of broken rice proteins under different pH (w=5 % at 25 °C) and mass fractions (pH=5.0 at 25 °C)

Parameters		PA/%				PP/%			
		Foaming capacity	Foaming stability			Foaming capacity	Foaming stability		
Time/min		0	20	60	90	0	20	60	90
pH	5.0	$(67.6\pm3.3)^{x}$	$(62.2\pm2.6)^{cx}$	$(41.5\pm1.8)^{bx}$	$(32.2\pm2.2)^{ax}$	$(62.5\pm2.6)^{x}$	$(58.0\pm2.9)^{bx}$	$(36.2\pm2.8)^{ax}$	$(29.2\pm2.0)^{ax}$
	7.0	$(73.3\pm1.7)^{x}$	$(65.9\pm2.3)^{bx}$	$(51.2\pm2.0)^{ay}$	$(45.9\pm2.6)^{ay}$	$(78.3\pm1.7)^{y}$	$(65.6\pm2.7)^{cx}$	$(43.1\pm2.2)^{bx}$	$(32.3\pm1.9)^{ax}$
	9.0	$(71.8\pm3.5)^{x}$	$(62.3\pm3.1)^{bx}$	$(47.6\pm1.9)^{axy}$	$(42.4\pm2.4)^{ay}$	$(71.1\pm3.6)^{xy}$	$(56.4\pm3.0)^{xc}$	$(41.2\pm2.6)^{bx}$	$(26.5\pm1.4)^{ax}$
w(protein)/%	1.0	$(38.3\pm1.7)^{x}$	$(23.2\pm1.6)^{cx}$	$(12.7\pm1.0)^{bx}$	$(8.6\pm0.6)^{ax}$	$(53.1\pm3.6)^{x}$	$(35.2\pm2.0)^{cx}$	$(18.0\pm1.2)^{bx}$	$(5.6\pm0.2)^{ax}$
	3.0	$(45.5\pm2.6)^{xy}$	$(33.2\pm1.2)^{bx}$	$(15.3\pm1.3)^{ax}$	$(10.1\pm1.3)^{ax}$	$(62.4\pm3.4)^{xy}$	$(43.1\pm2.0)^{cyz}$	$(19.1\pm1.2)^{ax}$	$(15.3\pm0.9)^{ay}$
	5.0	$(52.5\pm2.6)^{y}$	$(42.5\pm2.5)^{cy}$	$(21.3\pm1.6)^{by}$	$(13.7\pm1.5)^{ax}$	$(75.9\pm3.2)^{y}$	$(43.8\pm2.3)^{byz}$	$(25.1\pm1.4)^{ay}$	$(21.7\pm1.2)^{ay}$
	7.0	$(65.1\pm3.6)^{z}$	$(55.6\pm2.9)^{czxz}$	$(27.7\pm1.9)^{bz}$	$(18.2\pm1.4)^{ay}$	$(84.1\pm3.2)^{z}$	$(46.5\pm2.6)^{bz}$	$(23.6\pm1.1)^{axy}$	$(22.0\pm1.3)^{ay}$

Values superscripted with different letters in rows (a, b, c) and columns (x, y, z) are significantly different ($p<0.05$); values are means of triplicate determinations±S.D.

increased levels of added common bean flour. The cooking yields of sausages with PP were higher than those with PA at the same protein content.

Conclusions

Treatment of the broken rice with proteases generated protein hydrolyate products that had high protein cotent and appropriate functional properties, providing a convenient and inexpensive method to recover proteins from broken rice. The results suggest that the broken rice protein modified with alkaline protease or papain has a potential application in a variety of food products, particularly those requiring adequate water holding capacity, emulsifying properties and foaming capacity, and could be used as the protein fortification ingredient in a variety of food products for protein-deficient people in developing countries. The production of food products enriched with proteins could also add value to the broken rice, the low-cost by-product during milling.

Acknowledgements

This work was supported by the Programme of Imported Brains of Henan Technology University (Zhengzhou, PR China), Project No. 150288.

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