

# PROTEIN CHARACTERISTICS IN RELATION TO TEXTURAL AND PASTING PROPERTIES OF RICE AFTER STORAGE

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## Abstract

The protein characteristics of fresh and aged rice were investigated to correlate with the textural and pasting properties. The alkaline extractable proteins of aged rice were lower than those of fresh rice and they were extensively decreased in cooked rice. However, an increase in extractable proteins was noticed when the extraction buffer contained 1% sodium dodecyl sulfate, indicating that the rice proteins were stabilized via hydrophobic interaction. The extracted proteins from raw and aged cooked rice showed higher surface hydrophobicity and more disulfide bonds than those of fresh rice, suggesting more protein unfolding and higher disulfide linkages. In addition, aged rice proteins revealed higher molecular weight formed via disulfide bonds and hydrophobic interactions, as observed by sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After cooking, increased proglutelin and new aggregated protein (41 kDa) were observed. The harder texture and lower stickiness of aged cooked rice were noticed as compared with fresh cooked rice. Aged flour showed a lower breakdown, higher setback, and less paste viscosity, as monitored with a Rapid Visco Analyser. Removing protein from aged and fresh flour showed similar pasting curves, indicating that the proteins' characteristics had an influence on the pasting characteristics and were related to the cooked rice's texture.

**Keywords:** Proteins, hydrophobic interaction, disulfide bonds, pasting properties, textural properties

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## Introduction

The pasting and eating quality of rice normally changes after storage (Zhou *et al.*, 2003a). The change of texture is generally noticed after 6 months as it becomes harder and less sticky. The pasting properties of aged rice monitored with a Rapid Visco Analyser (RVA) showed a higher peak viscosity and setback but lower breakdown (Teo *et al.*, 2000; Sowbhagya, and Bhattacharya, 2001; Zhou *et al.*, 2003a; Park *et al.*, 2012). In addition, the rice proteins aggregated and their molecular size increased through the disulfide bonds after storage for 1 year (Chrastil and Zarins, 1992; Chrastil 1993). However, these studies of proteins were conducted on raw rice.

Most of rice's proteins are glutelin or oryzenin, a storage protein with a high molecular weight of 64-500 kDa (Sugimoto *et al.*, 1986), which strongly binds to starch granules via disulfide and/or hydrophobic interaction (Lim *et al.*, 1990). Several studies have demonstrated that rice proteins are related to pasting properties by the disruption of proteins. The addition of dithiothreitol,  $\beta$ -mercaptoethanol (BME), and protease changes the pasting profile of rice flour (Hamaker and Griffin, 1990, 1993; Zhou *et al.*, 2003a). Zhou *et al.* (2003a) reported that the BME addition showed an increased peak viscosity and the protease addition also showed an increase in peak viscosity, breakdown, and final viscosity of flour stored at 37°C for 16 months. Teo *et al.* (2000) found that adding isolated glutelin to rice starch showed a higher peak viscosity and breakdown which was close to flour stored at 35°C for 14 week. Moreover, the protein network phase of cooked rice grains showed the bi-continuous structure of the proteins and starch but it was different in terms of the structural arrangement between fresh and aged cooked rice (Likitwattanasade and Hongsprabhas, 2010). These findings imply that the changes in rice's properties are associated with the proteins' structures.

Not only is the disulfide bond important in rice proteins during storage but the solubility and surface hydrophobicity could also evaluate the physicochemical and functional properties of the proteins. Ju *et al.*

(2001) found that the higher surface hydrophobicity of glutelin was induced by heating at 65-95°C. Glutelin is low water soluble due to the hydrophobic interaction, hydrogen bond, and disulfide bonding (Hamada, 1996). After rice storage, the solubility of glutelin and prolamin was decreased (Zhou *et al.*, 2003b). Most studies on rice storage have focused on the individual changes of the pasting and textural properties and the proteins' structures. Limited information is available on the relationships between the rice proteins' characteristics including the physicochemical properties, structure, chemical bonding, and pasting properties of aged and fresh rice, especially in cooked rice. In order to understand the role of proteins during rice storage, the aim of this study was to investigate the properties of rice proteins in those aspects in both raw and cooked rice in relation to the pasting and textural properties of rice.

## Materials and Methods

### Materials

Newly harvested and 1-year aged (stored in a sack at 27±°C) rough rice samples (Khaw Dawk Mali 105 cultivar) were purchased from Ubon Ratchathani Rice Research Center, the Bureau of Rice Research and Development, Thailand. The samples were dehulled and polished with a THU 35 B testing husker (Satake Corp., Hiroshima, Japan). Some portions of the grains were milled using a M20 Universal mill (IKA Werke GmbH & Co. KG, Staufen, Germany) connected to a cooling bath for controlling the samples' temperature of 15°C and then sieved through a 140-mesh sieve. Rice starch was prepared using the modified method of Ju *et al.* (2001). All chemicals were of an analytical grade.

### Chemical Composition

The moisture was determined in accordance with the AOAC official method (AOAC, 1995). The protein content was determined by the Kjeldhal nitrogen method (AOAC, 2000) ( $N \times 5.95$ ) and the fat content determined with Soxhlet extraction using a 2050 Soxtec Auto Extraction System (Foss A/S, Hillerød, Denmark). Amylose was

analysed by the iodine-colorimetric method according to Juliano *et al.* (1981).

### Rice Cooking

Cooked rice was prepared using a modified method of Singh *et al.* (2005). Twenty grams of rice grains was suspended with 30 mL of distilled water for the fresh rice samples or 36 mL for the aged rice samples. The samples were loaded into an aluminium can (6.0 cm diameter) and covered. All samples were held for 10 min and then cooked in a boiling bath with a minimum cooking time of 21 min for the fresh rice and 22 min for the aged rice. The cooked samples were held at room temperature for 4 h before textural measurement.

### Protein Extraction

Raw rice proteins were extracted by a modified method from Ohno *et al.* (2007). Rice flour (1 g) was mixed with 20 mL of 10 mM sodium hydroxide (NaOH) containing 1 or 5% of sodium dodecyl sulfate (SDS). The suspension was mixed at room temperature for 1 h with a shaking speed of 250 rpm and then centrifuged at 10000xg for 15 min. The extracted solution was analysed for protein content by the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as a standard.

One gram of cooked rice was mixed with 3 mL deionized (DI) water in a centrifuge tube. The suspension was equilibrated at 37°C for 15 min prior to adding 1 mL of 0.05%  $\alpha$ -amylase from porcine pancreas (EC 3.2.1.1) (Sigma-Aldrich Corp., St. Louis, MO, USA). The mixture was incubated at 37°C for 1 h in a shaking water bath. Then, it was mixed with 0.05 mL of 1.0 M NaOH and SDS was added to obtain a final concentration of 1 or 5% of SDS, respectively, and the total volume was adjusted to 5 mL with DI water. The mixture was mixed in a shaker at room temperature for 1 h at 250 rpm and centrifuged at 10000xg for 15 min. The protein content of the extracted solution was determined by the Lowry method (Lowry *et al.*, 1951).

### Surface Hydrophobicity

The surface hydrophobicity ( $S_0$ ) of the alkaline crude protein extracts was determined using a hydrophobic fluorescence probe,

1-anilino-8-naphthalenesulfonate (ANS), according to the method of Paraman *et al.* (2007) and Kato and Nakai (1980) with slight modifications. Protein solutions (0.2% w/v, on protein basis) were prepared using a 50 mM carbonate/bicarbonate buffer at pH 10. Each extracted protein sample was diluted with the buffer for 5 protein concentrations (0.01 to 0.1% w/v). A volume of 2 mL of each diluted sample was added to 10  $\mu$ L of ANS (8 mM of ANS in 0.1 M potassium phosphate buffer pH 7.0) and mixed immediately. The mixtures were held in the dark for 10 min. The fluorescence intensity (FI) of the protein extracts was measured using a spectrofluorophotometer (Shimadzu RF-540, Shimadzu Corporation, Kyoto, Japan) at 340 nm (excitation) and 470 nm (emission). The excitation and emission width slits were set at 10 nm and measured in the low intensity mode. The buffer solution with the ANS probe was used as a buffer blank. The buffer with each protein dilution was measured for sample blanks. The net FI was calculated by subtracting the FI of the buffer blank and each sample blank from the FI of each of the diluted protein samples with a probe. The surface hydrophobicity was expressed as the slope by plotting of the net FI versus the protein concentration.

### Reactive Sulfhydryl and Disulfide Analysis

The total sulfhydryl content (SH) was measured with Ellman's reagent (5,5'-dithiobis (2-nitrobenzoic acid (DTNB)) according to a modified method described in Yongsawatdigul and Park (2003). The protein solution (500  $\mu$ L) was mixed with 4.5 mL of 0.5 M Tris-HCl buffer (pH 8.0) containing 10 mM ethylenediaminetetraacetic acid (EDTA) and 8 M urea. The protein mixture (2 mL) was added to 0.2 mL of 0.1% DTNB. Then, it was incubated in a water bath at 40°C for 25 min. The absorbance was read at 412 nm. DI water was used as a blank. The reactive SH group was calculated using an extinction coefficient of 13600 M<sup>-1</sup>cm<sup>-1</sup>.

The disulfide bond content was calculated by the subtraction of the reactive SH group from the total sulfhydryl group (SH+reduced disulfide (SS)). The measurement of the total SH group was slightly modified, as described by Pfeiffer and Metzler (1996) and

Thannhauser *et al.* (1984). The protein solution (500  $\mu$ L) was mixed with 4.5 mL of the 0.5 M Tris-HCl buffer (pH 9.5) containing 100 mM sodium sulfite, 10 mM EDTA, and 8 M urea. Two mL of the mixture was added with 20  $\mu$ L of 5 mM of disodium 2-nitro-5-thiosulfobenzoate (NTSB), and mixed immediately. The mixtures were incubated at 40°C for 25 min. The absorbance was measured at 412 nm. The total sulfhydryl group was calculated using an extinction coefficient of 13600 M<sup>-1</sup>cm<sup>-1</sup>.

### **Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

Aggregation and separation of the rice proteins after storage were studied by SDS-PAGE according to the method of Laemmli (1970) using a Bio-Rad Mini-Protein II cell (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Stacking gel and separated gel were prepared at 4% and 12% of polyacrylamide, respectively. The alkaline crude protein extract was mixed with a buffer (0.125 M Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, and 0.002% bromophenol blue) with a reducing agent (10%  $\beta$ -mercaptoethanol (BME)) and without a reducing agent with a protein solution-to-buffer ratio of 1:1. Then, it was heated in a boiling bath for 5 min and loaded into the gel (25  $\mu$ g from raw rice and 15  $\mu$ g from cooked rice). The protein samples were separated at a constant voltage of 120V. Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to stain the proteins. The destaining solution contained 25% of ethanol and 10% of acetic acid. The standard marker proteins were rabbit myosin (205 kDa),  $\beta$ -galactosidase (116 kDa), rabbit phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (20 kDa),  $\alpha$ -lactalbumin (14 kDa), and aprotinin (6.5 kDa).

### **Textural Properties**

The texture of cooked rice in the aluminium can was analyzed using a TA-Xtplus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) equipped with a 3.5 cm cylinder probe. The samples were compressed to a depth of 12 mm, and the pretest, test speed, and posttest speed were 1 mm/s. The resulting force-distance curve

was used to acquire the textural parameters which were hardness (g) and stickiness (g<sup>-1</sup>). The hardness is the maximum peak force of the first compression of the cooked rice samples and the stickiness is the maximum force of the second peak. All samples were analysed in 5 replications.

### **Pasting Properties**

A Rapid Visco Analyser (Rva-3D, Newport Scientific, Inc., Irvine, CA, USA) was used to determine the pasting properties of the samples. The rice flour or starch samples (3 g, 14% moisture) were mixed with DI water (25 mL). The initial speed rotation was 960 rpm/min for 10 sec before the measuring step at 160 rpm/min. The measurement settings were the heating step from 50°C to 95°C in 3 min 42 sec, holding at 95°C for 3 min 30 sec, cooling from 95°C to 50°C in 3 min 48 sec, and holding at 50°C for 1 min. The viscosity parameters of peak viscosity (PV), final viscosity (FV), breakdown (BD), and setback (SB) were expressed in rapid viscosity units (RVU). All tests were analysed in 3 replications.

### **Statistical Analysis**

Analysis of variance was performed using SPSS version 12.0 for Windows (SPSS Institute, Inc., Cary, NC, USA). Comparison of means was conducted using Duncan's multiple range tests. The experiments were run at least in triplicate.

## **Results and Discussion**

### **Protein Solubility**

The crude protein contents of the aged and fresh rice were not different ( $p \geq 0.05$ ) (Supplementary Table 1) but the proteins of the aged rice were less solubilized in the alkaline solution as compared with the fresh rice (Table 1). After using the alkaline solution containing 1% SDS, a higher yield of protein extract from both types of rice was obtained. SDS is an anionic detergent which can disrupt the hydrophobic interaction of proteins and transform them into soluble forms. A 3-dimensional structure of proteins was stabilized by hydrophobic interaction (Jittinandana *et al.*, 2003). From the results, it was presumed that stronger hydrophobic interactions and

a more stabilized protein structure occurred in the aged rice. This result is consistent with Mujoo *et al.* (1998) who reported that more proteins could be extracted from raw, roasted, and flaked rice samples when extracted with 1% SDS solution. Villareal *et al.* (1976) demonstrated that the 0.6%  $\beta$ -mercaptoethanol solution containing 0.5% SDS could extract up to 94-98% of proteins in rice. However, the aged protein extraction with SDS was lower than that of the fresh rice ( $P < 0.05$ ).

When compared with raw rice, the proteins of cooked rice were less soluble. After extraction with 1% SDS, the proteins from cooked rice were more soluble. Therefore, the heating process induced more hydrophobic interaction of the proteins. However, the soluble protein extraction with alkaline containing SDS from cooked rice was still less than that from raw rice for both fresh and aged rice. This is in agreement with the finding of Bruneel *et al.* (2010) who reported that the SDS extractable proteins in all spaghetti products were decreased after cooking. The

lesser extraction from the SDS solution may be related to more polymerization of proteins with other bonds/interaction during cooking.

For the cooked rice samples, the amounts of proteins extracted from both aged and fresh cooked rice were not different ( $P < 0.05$ ); this differs from the former experiment (no addition of  $\alpha$ -amylase to hydrolyze starch gel) and this experiment showed the lower amount of proteins extracted from aged cooked rice when compared with fresh cooked rice (data not shown). This result is associated with those observed in raw rice in Table 1. It was likely that the proteins of aged cooked rice have a stronger interaction with the gel of starch polymers than those in fresh cooked rice. In addition, the proteins of cooked rice were also more soluble in the alkaline solution with the 1% SDS solution, indicating that the heating process induced the hydrophobic interaction of rice proteins. Moreover, hydrophobic interaction is an important force related to protein solubility in cooked rice.

**Supplementary Table 1. Chemical composition of milled rice**

Samples	Proximate composition (%)			
	Moisture	Crude fat	Crude protein	Amylose
Fresh rice	10.67 $\pm$ 0.12a	0.40 $\pm$ 0.028a	7.94 $\pm$ 0.15a	18.88 $\pm$ 0.10a
Aged rice	9.44 $\pm$ 0.43b	0.40 $\pm$ 0.038a	7.68 $\pm$ 0.04a	19.84 $\pm$ 0.23a

The different letters in the same column indicate the significant differences ( $p < 0.05$ ).

**Table 1. Alkaline extractable proteins and physicochemical properties of alkaline extractable proteins from raw and cooked rice**

	Raw rice		Cooked rice	
	Fresh	Aged	Fresh	Aged
<b>Alkaline extractable protein</b>				
<i>(mg/g sample)</i>				
0% SDS	66ax	30bx	24ay	24ay
1% SDS	86ax	69bx	44ay	45ay
<b>Physicochemical properties</b>				
Surface hydrophobicity	598by	691ay	1577bx	2418ax
Total SH ( $\mu$ mole/g protein)	15ay	16ay	62bx	83ax
SS ( $\mu$ mole/g protein)	49by	59ay	100bx	129ax

The different letters (a-b) in the same row indicate the significant differences between fresh and aged rice ( $p < 0.05$ ). The different letters (x-y) in the same row indicate the significant differences between raw and cooked rice ( $p < 0.05$ ).

### Physicochemical Properties of Proteins Surface Hydrophobicity

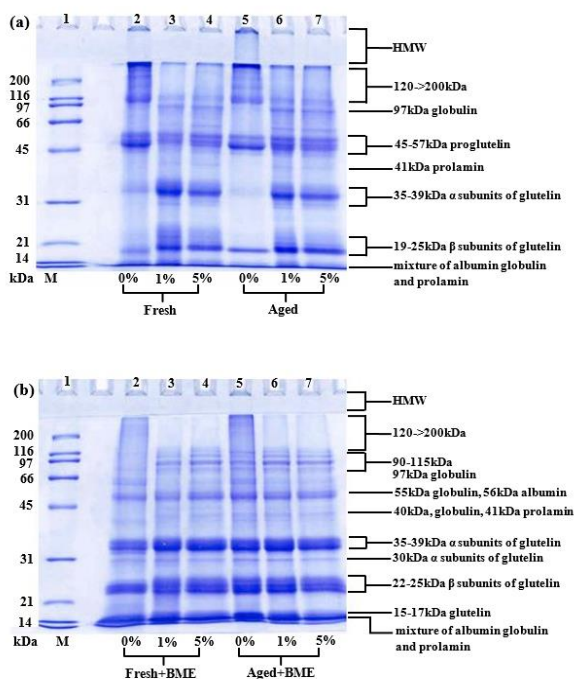
Surface hydrophobicity ( $S_0$ ) of proteins extracted from the raw aged rice was higher than that of fresh rice (Table 1). The high  $S_0$  suggested that the more the proteins unfolded, the more the hydrophobic group was exposed. This change caused the higher insolubility of proteins (van der Borgh *et al.*, 2006). These results could explain the fewer extractions of proteins in aged rice. An increase in exposure of the hydrophobic side chains resulted in changes in protein conformation (Privalov *et al.*, 1986; Franks, 1995). Therefore, the protein conformation of aged rice was different from that of fresh rice. The unfolding of heat-induced proteins increased the surface hydrophobicity of the fresh and aged rice by 2.6 and 3.5 times, respectively. This result was in agreement with Tang *et al.* (2002) who reported that the  $S_0$  of rice glutelin was increased after heating at 60°C which was similar to Ju *et al.* (2001). An increase in  $S_0$  after cooking facilitated protein

linkages via hydrophobic interaction in the cooked rice. Similarly, the  $S_0$  in the aged cooked rice was also higher than the fresh cooked rice. Therefore, heating enhanced the degree of  $S_0$  of proteins in the rice.

### Disulfide Bond and Reactive SH Content

The disulfide bond and reactive SH contents are shown in Table 1. For raw rice, the disulfide content of aged rice was higher than for fresh rice ( $p < 0.05$ ). The reactive SH amount of aged and fresh raw rice was low. Therefore, the proteins in aged rice were more aggregated via the disulfide bond than those in fresh rice.

The cooking process has the effect of promoting the disulfide and reactive SH contents in rice protein. The higher disulfide content occurring in cooked rice was associated with increasing the surface hydrophobicity and insoluble proteins, especially proteins in aged cooked rice. Many studies have reported that thermal processing promoted the formation of the disulfide bond, consequently reducing the



**Figure 1.** SDS-PAGE patterns of alkaline extractable proteins from fresh and aged rice with 0, 1, and 5% SDS; (a) non-BME treatment buffer, and (b) BME treatment buffer. M = standard marker proteins

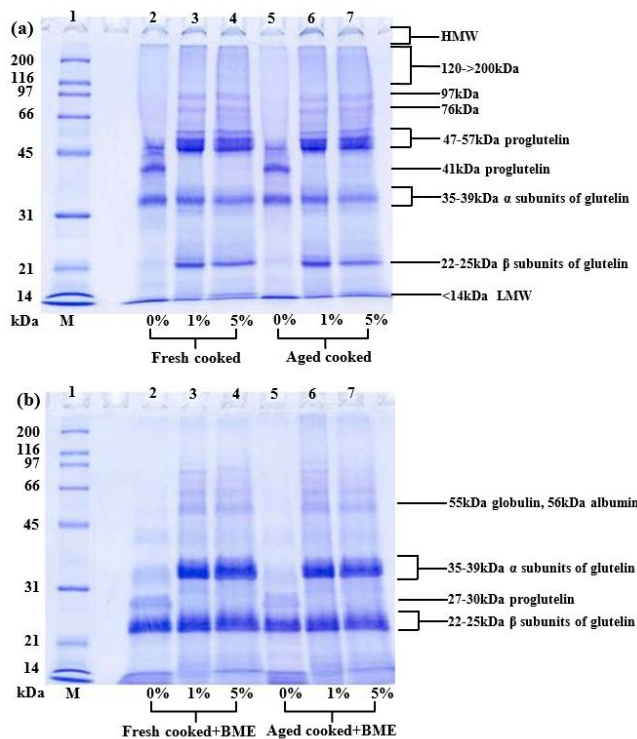
protein solubility of wheat, sorghum, maize, and soy proteins (Dexter and Matsuo, 1979; Hager, 1984 and Ummadi *et al.*, 1995). Moreover, the reactive SH of aged and fresh rice was also increased after cooking. This could bring about the tendency of cross-linking in proteins. The disulfide and reactive SH contents are the indicators of the degree of protein polymerization and aggregation (Jittinandana *et al.*, 2003). The greater number of disulfide linkages showed the more rigid proteins by inducing the restriction of bond rotation (Torchinsky, 1981) that stabilized protein aggregation (van der Plancken *et al.*, 2005). Thus, proteins in aged cooked rice would be aggregated and stabilized more by the disulfide bond than those in fresh cooked rice

#### SDS-PAGE of Proteins from Rice Flour

The SDS-PAGE patterns of rice proteins from aged and fresh rice are shown in Figure 1(a). Four major protein bands, the  $\beta$ -subunits of glutelin (19 - 25 kDa),  $\alpha$ -subunits of glutelin

(35 - 39 kDa), proglutelin (45 - 57 kDa), and 120 -> 200 kDa were noticed in the fresh and aged rice proteins extracted with NaOH. The molecular weight of glutelin subunits depends on the rice cultivar, protein extraction method, and molecular characterization. Likitwattanasade and Hongprabhas (2010) reported that the molecular weight of the major protein subunits of glutelins were around 20, 30, and 50 kDa. Chrastil and Zarins (1992) observed the major proteins of 22 - 23 kDa and 32 - 37 kDa. Wen and Luthe (1985) found that the acidic ( $\alpha$ ) and the basic ( $\beta$ ) polypeptides of rice glutelin had molecular weights ranging from 28.5 to 30.8 kDa and 20.6 to 21.6 kDa, respectively. Van der Borgh *et al.* (2006) reported that the  $\beta$ -subunits had 25 kDa and the  $\alpha$ -subunits had 32 kDa.

The aged rice proteins (lane 5) showed lower amounts of proteins of 120->200 kDa proglutelin,  $\beta$ -subunits, and  $\alpha$ -subunits of glutelin appearing as a weak intensity band than those of the fresh rice proteins (lane 2);



**Figure 2.** SDS-PAGE patterns of alkaline extractable proteins from fresh and aged cooked rice with 0, 1, and 5% SDS; (a) non-BME treatment buffer, (b) BME treatment buffer. M = standard marker proteins

meanwhile, there was a large amount of high molecular proteins in which the high molecular weight on the stacking gel of aged rice showed a higher intensity when compared with the fresh rice proteins. This suggested that the aggregation of aged rice proteins was more pronounced than that of fresh rice proteins. This result is consistent with Chrastil and Zarins (1992) who revealed that the low molecular weight peptide subunits of glutelin protein from aged rice stored at 40°C decreased while the high molecular weight peptide subunits increased. Similarly, Ohno *et al.* (2007), reported the decreased small molecules (21 and 32 kDa) and increased large molecules (48, 99, and 170 kDa) of proteins from aged japonica rice stored at 30°C for 5 months. It could be deduced that the high amount of the large proteins could be related to the lower solubility of proteins in aged rice when compared with fresh rice

The high molecular weight proteins on the stacking gel did not remain and 120 -> 200 kDa was decreased while the  $\beta$ - and  $\alpha$ -subunits were increased when using 10 mM NaOH containing 1 or 5% SDS (lanes 3, 4, 6, and 7). In a comparison between the aged and fresh rice, the greater intensity of proglutelin and 120 -> 200 kDa and the lesser intensity of the  $\beta$ - and  $\alpha$ -subunits were noticed in the aged rice proteins (lanes 6 and 7) in alkaline-SDS extract solution. These results implied that the large proteins existing in aged rice were generated from an aggregation of the small molecules of proteins via hydrophobic interaction.

When proteins were treated with BME, the high molecular weight proteins in the stacking gel were not noticed (lanes 2 and 5 in Figure 1(b)). The proglutelins disappeared for

all lanes while the  $\beta$ - and  $\alpha$ -subunits were intensified. When combined with the above result, it suggested that the high content of proglutelins including the large proteins (in the stacking gel region) appearing in aged rice resulted from the  $\beta$ -subunits and  $\alpha$ -subunits being linked via the disulfide bonds. From the results of SDS-PAGE, it could be concluded that the increased large proteins resulted from the aggregation of glutelin protein subunits by disulfide linkages and hydrophobic interactions after storage.

### SDS-PAGE of Protein from Cooked Rice

Figure 2 displays the SDS-PAGE of proteins from cooked rice extracted with and without SDS. The pattern of proteins changed after cooking as compared with the raw rice. The band of proteins more than 66 kD was barely observed in lane 2 and lane 5 (Figure 2(a)). Furthermore, the  $\alpha$ -subunit band showed a high intensity but the  $\beta$ -subunits disappeared. This result is associated with the reduction of protein extraction. Thus, the bonds of 35-45 kDa were only noticed. In addition, the new protein band of 41 kDa appeared in a buffer without SDS. After being extracted with the alkaline-SDS solution, the  $\beta$ -subunit was present but the 41 kDa proteins vanished. It implied that the 41 kDa derived from the  $\beta$ - $\beta$  subunit association via hydrophobic interaction which was not found in raw rice. Moreover, the proglutelin band (47 - 57 kDa) (lanes 3, 4, 6, and 7) showed a higher intensity with SDS addition when compared to the non-SDS protein extraction (lanes 2 and 5) that was different from the raw rice pattern. It is probably due to either the breakdown of

**Table 2. Pasting properties of flour and starch from fresh and aged rice**

RVA parameters	Fresh		Aged	
	Flour	Starch	Flour	Starch
PT (°C)	75ay	72bx	89ax	71by
PV (RVU)	148ax	144bx	126by	132ay
BD (RVU)	69ax	71ax	31by	54ay
FV (RVU)	133ay	106by	176ax	116bx
SB (RVU)	54ay	33by	82ax	40bx

The different letters (a-b) in the same row indicate the significant differences between flour and starch ( $p < 0.05$ ). The different letters (x-y) in the same row indicate the significant differences between fresh and aged samples ( $p < 0.05$ ).



aggregated proteins or the subunit aggregations being induced in cooked rice.

In the presence of BME, the proglutelins' band vanished (Figure 2(b)), suggesting that the new proglutelins which developed after cooking probably resulted from the  $\alpha$ -subunit (25 - 39 kDa) and  $\beta$ -subunit (22 - 25 kDa) linking via disulfide bonds. The pattern of all protein samples was not different, probably because some of the proteins remained in the starch sediment. Although the pattern of proteins between the aged and fresh cooked rice is not different, the orientation and/or configuration of proteins may not be similar.

### Pasting Properties

As compared with native rice flour, the pasting temperature (PT), final viscosity (FV), and setback (SB) of starch were decreased, but the breakdown (BD) was increased ( $p < 0.05$ ), as demonstrated in Table 2. The reduction of the PT implied that the starch granules were easily swollen, which is similar to the result of Likitwattanasade and Hongprabhas (2010). An increase in the BD of the starch samples suggested the destabilization of the starch granules (Liang and King, 2003) that were less resistant to shear force, leading them to break easily. Regarding this result, it indicates that proteins have an influence on delaying the swelling and breaking down of starch.

Moreover, the decreased FV of starch could be associated with the proteins' matrix. As it was removed, the gel structure was not supported, resulting in a low viscous paste. This suggested that proteins play an important role in the pasting properties of starch. In addition, the peak viscosity of aged starch was lower when compared with the fresh starch. This result was different from Zhou *et al.* (2003b) who reported a similar peak viscosity for aged and fresh isolated starches. It indicates that the change of rice gel properties after storage has a combined effect on starch and proteins.

In a comparison between flour and starch, the aged rice flour showed a greater change than fresh rice flour. The PT, FV, and SB of aged flour reduced by 20, 34, and 50% while fresh rice starch decreased by 4, 20, and 39%, respectively. This was associated with more insoluble proteins, higher  $S_0$ , and more disulfide content in aged rice. Furthermore, during the cooling step of the RVA measurement, the pasting curves of aged and fresh rice starch nearly overlapped (Supplementary Figure 1). This result was concomitant with Hamaker and Griffin (1990) who found that the Brabender viscosity of cooked rice treated with protease was reduced at all points in the viscogram.

Aged rice flour showed a higher PT but lower PV and BD than fresh rice flour (Table

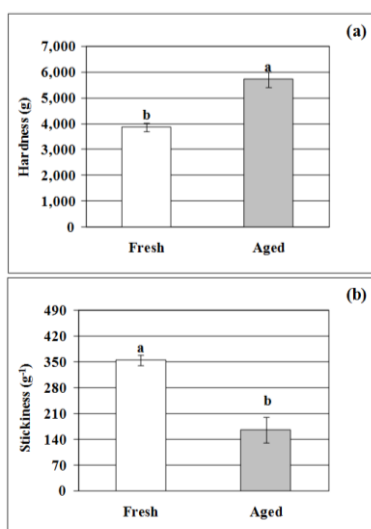


Figure 3. Texture of cooked fresh and aged rice; (a) hardness and (b) stickiness

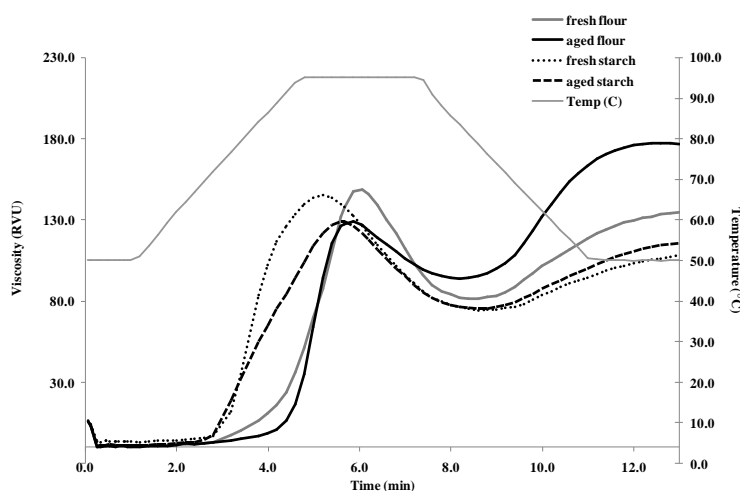
2). It indicated that the integrity of the starch granules was greater in aged rice, and consequently was more resistant to swelling and had less capacity to rupture (Noomhorm *et al.*, 1997). The rice structure became progressively organized during storage (Sowbhagya and Bhattacharya, 2001), resulting in a low hot paste viscosity. This result is related to the increased hydrophobic interaction and disulfide content, inducing more aggregation of proteins in aged rice that could protect the starch granules from swelling and breaking. In addition, the FV of aged rice flour was also high. This is likely due to the interaction between the starch remnant and the large proteins, resulting in the high viscosity and consequently hard gel formation as compared with the fresh rice.

These results suggested that proteins had a predominant effect on the pasting properties of rice. Therefore, study of the proteins' characteristics before and after rice storage would permit more understanding of the changes in the starch paste and cooked rice texture.

### Cooked Rice Texture

Hardness and stickiness are important parameters to evaluate the texture of cooked rice. The hardness of aged rice was higher, whereas the stickiness was lower when

compared with the fresh rice. This is commonly recognized and in agreement with other reports (Consuelo *et al.*, 1981; Juliano, 1985; Tamaki *et al.*, 1993; Tran *et al.*, 2005; Michiko *et al.*, 2015). Although the texture changed after storage for 1 year, the amount of crude protein, fat, and amylose contents were not different ( $p > 0.05$ ) (Supplementary Table 1). Since the pasting properties of aged flour which hardly swelled and showed a higher cold paste viscosity are relevant to the harder texture of aged cooked rice, the proteins' characteristics could be an important factor relating to the texture of aged rice. From the above results, more unfolded proteins and an increase in the  $S_0$ , hydrophobic interaction and disulfide content were observed and are concomitant with the high amount of large size proteins. Proteins were highly accumulated near the outer layer of the rice grains (Saito *et al.*, 2010) and formed 3-dimension networks with a honeycomb-like structure in cooked rice grains (Likitwattanasade and Hongsprabhas, 2010). It was possible that the protein polymerization of aged rice was induced by hydrophobic interaction and disulfide bonds near the outer layer of the rice grains, consequently decreasing water penetration to the grains. Then, the texture of aged cooked rice was harder.



Supplementary Figure 1. Pasting profiles of fresh and aged rice flours or starches

## Conclusions

After storage, rice proteins aggregated through the disulfide bond and hydrophobic interaction that was related to the changes of solubility and physicochemical properties. After cooking these properties changed, including reduced protein solubility, higher surface hydrophobicity, and more disulfide content which was more pronounced in aged rice. Furthermore, the more aggregated proteins were associated with the increased high molecular weight proteins that may prevent water absorption of starch granules that have an influence on the pasting properties with higher final viscosity, consequently resulting in the harder texture of aged rice.

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