Storage time affects storage proteins and volatile compounds, and pasting behavior of milled rice

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Abstract

Albumin, globulin, glutelin and prolamin fractions were isolated from a Thai rice variety (cv. Khao Dawk Mali 105), which was stored at 25°C for 0-7 months and characterised by yield and protein contents. The solubility of protein under the influence of storage time was studied by extractable protein contents, while pasting properties of rice flour following storage of the grain for up to 7 months were investigated by rapid viscosity analysis (RVA). The total protein content of fresh harvest rice was decreased 19.81%. The highest contents of glutelin and globulin were 70% and 20%, respectively, while albumin and prolamin were found in relatively equal amount in rice grain. Glutelin was most significantly affected on pasting properties, while globulin affected on peak viscosity and also setback and breakdown properties, but on the lesser extent than glutelin. Albumin and prolamin affected mostly on setback. The results showed that each type of the storage proteins affected on viscosity of pasting properties of rice starch during storage. The results of viscograms from the samples provided a valid comparison of the effects of aging on pasting behavior and confirmed that each type of the stored-rice proteins affected on viscosity of pasting properties of rice starch during storage. Stored-rice protein contents had a significant positive correlation with 2-acetyl pyrroline content and a negative correlation with hexanal contents. Shorter storage time was considered to be used to preserve 2-acetyl pyrroline and stored-rice protein of the aromatic rice. Stored-rice proteins had some effects on viscosity properties of rice flour so they could be important for food applications.

Keywords: protein, rice, pasting property, acetyl pyrroline, hexanal

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Introduction

Cereal grains can be stored for long periods without microbial spoilage, however, biochemical changes do occur during aging. The grain respires, dry matter is lost, and functional and nutritional aspects of the grain are altered (Reed, 1992). Whole rice grain contains many types of proteins which have been isolated and characterised, mainly according to their solubility properties, using the Osborne extraction method (Marshall, 1994). Proteins are found in different parts of the rice grain including the endosperm, the polish and the bran, most being within the endosperm (storage proteins) cells, situated in protein bodies between the starch granules (Lasztity and Salunkhe, 1979). Main cereal proteins can be classified into albumins, globulins, prolamins, and glutelins fractions. While glutelins and prolamins are the majoritary proteins in wheat and corn, prolamins are very scarce (2%) in rice, being glutelins the most abundant proteins in this cereal followed by albumins (11%) (Belitz and Grosch, 1998). Because of its abundance, rice glutelin has been extensively studied in biochemical and molecular genetic investigations (Okita et al., 1989). During storage of rice, the molecular weight of glutelin increases significantly, which correlates with an increase in disulphide bonding (Chrastil, 1990). The decrease in solubility is thought to explain the decrease in stickiness observed in stored rice (Chrastil, 1994). Since proteins impact so much on the end-use of other cereals, it is most likely that they contribute to the quality of cooked rice.

However, studies on rice proteins have been limited when compared to those of the starch component. Therefore, it is important to be able to measure the contribution of proteins to rice quality.

During storage, the flavour of rice can deteriorate due to changes in its volatile components by way of several mechanisms. These include breakdown of desirable volatile constituents, losses via diffusion out of the rice into the environment and generation of undesirable volatile materials by one or more of a number of mechanisms. This is of particular importance for fragrant rices in which the unique aroma characteristics rely on the relative proportions of many individual components.

The purposes of this paper are to present the effects the storage on the solubility of storage proteins of the rice grain and to investigate the effect of storage proteins removal on the pasting properties of rice flour before and after storage and recognizable aroma description was obtained for 2-acetyl-1-pyrroline (2-AP) and hexanal of the stored rice.

Materials and Methods

Rice samples

One hundred kilos of the paddy rice Khaw Dawk Mali 105 were provided by the Rice Research Center, Phathumthani province. The bags were place at 25°C. Samples were analyzed physicochemical properties of rice. Fresh harvest and 7-month stored paddy rice were for albumin, globulin, glutelin and prolamin contents and physicochemical properties.

Physical analysis

The color of brown rice was determined by Minolta Model DP-301 colorimeter. Color values (L, a, and b) were measured. A white standard tile was used to calibrate the colorimeter (L= 100.01, a= -0.01, b= -0.02) before measurements. Therefore L measures lightness (luminosity) and varies from white to black. The chromatically (a and b values) gives designations of color as follows; a-value measures redness when positive, gray when zero, and greenness when negative,

b-value measures yellowness when positive, gray when zero, and blueness when negative.

Isolation of proteins

Proteins were extracted from rice flour based on their solubility at room temperature (25°C) in water, 5% NaCl, 0.1 M NaOH and 70% ethanol using the procedure of Ju et al. (2001). Milled rice flour (100 g) was defatted with 400 ml hexane and dried. The defatted flour was then extracted with 400 ml distilled water and centrifuged at 3000g for 30 min to obtain the albumin fraction (supernatant). The residue was extracted with 400 ml of 5% NaCl to obtain the globulin fraction. The residue after extraction of globulin was extracted with 0.1 M NaOH (1 h) to obtain the glutelin fraction, while the residue after glutelin extraction was extracted with 70% ethanol to obtain the prolamin fraction.

Rapid viscoanalysis (RVA)

The pasting properties of the various samples were determined with a Rapid Visco Analyser. Rice flour was slurried with distilled water. The temperature profile involved an initial 10 s high-speed stir that dispersed the sample prior to the beginning of the measuring phase at 160 rotations/min. Temperature was held at 50°C for 1 min and then raised to 95 °C in 3.75 min, held for 2.5 min, cooled to 50°C in 3.75 min, and held for 5 min. Values are reported in min, °C or rapid viscoanalyser units (RVU).

Sample extraction

The extraction vials used were 12 x 32 mm with a TFE septa and crimp top. Extractions were performed using 0.3 g of ground rice in 0.5 ml of stock solution. The stock solution consisted of methylene chloride with 0.459 ng/ml of 2, 4, 6-trimethylpyridine used as a standard.

Gas Chromatography

Method development and sample analysis were performed on a gas chromatograph (GC) and flame-ionization detection (FID). After optimization, the GC operating parameters were chosen: injector 155°C; detector 300°C; initial temperature 35°C; initial time 1 min, first rate 9 °C/min to 120°C, second rate 25°C/min to 275°C; final temperature 275°C; final time 2 min. Helium was the carrier gas and was set at a constant flow rate of 7.2 ml/min. The time required to analyze the peaks of interest and purge and cool the system was 25 min. The 2-AP peak was originally identified by chromatography coupled aroma perception with simultaneous FID similar to the procedure described by Tanchotikul and Hsieh (1989). Analytical reagent-grade hexanal was used for its verification.

Results

The b values represented yellow color of the paddy, brown and milled rice during storage time and temperature at 25°C is shown in Figure 1. The results showed b value increased according to storage and the b value at 37°C was higher than the storage at 25°C. As shown in Figure 1, there was increase in b value after storage at 25°C and 37°C for 4 months and 3 months, respectively.

Isolation of rice protein fractions

Of the four protein components, glutelin represented approximately 80% of total protein content in fresh harvest rice, followed by globulin, while albumin and prolamin contents were nearly the same. Table 1 shows the effect of isolation methods on the yield and protein content of the rice protein fractions of fresh harvest rice and 7-month stored rice. For fresh

harvest rice, the major fraction (about 72% of total) was glutelin which also had the highest protein concentration. The globulin fraction was next at around 19% yield followed by albumin (5%) and prolamin (4%).

Table 1 shows the effect of isolation methods on the yield and protein content of the rice protein fractions of 7-month stored rice. The major fraction (about 69% of total) was glutelin which also had the highest protein concentration. The globulin fraction was next at around 17% yield followed by prolamin (8%) and albumin (3%). In the extraction, the albumin fraction had the lowest in protein content compared with the globulin, prolamin or glutelin fractions.

There was a marked difference in prolamin content, containing 7.9% for stored rice and 4.34% for fresh harvest rice, with the relative difference being higher than 45% and there was also difference in albumin content, containing 4.7% for stored rice and 2.79% for fresh harvest rice, with the relative difference being higher than 40%.

On the other hand, there was little difference in globulin and glutelin content among fresh harvest rice and stored rice with the relative difference being lower than 12%. Although albumin and prolamin contents were lower in the rice, their relative difference between fresh harvest rice and stored rice was approximately 40-45%.

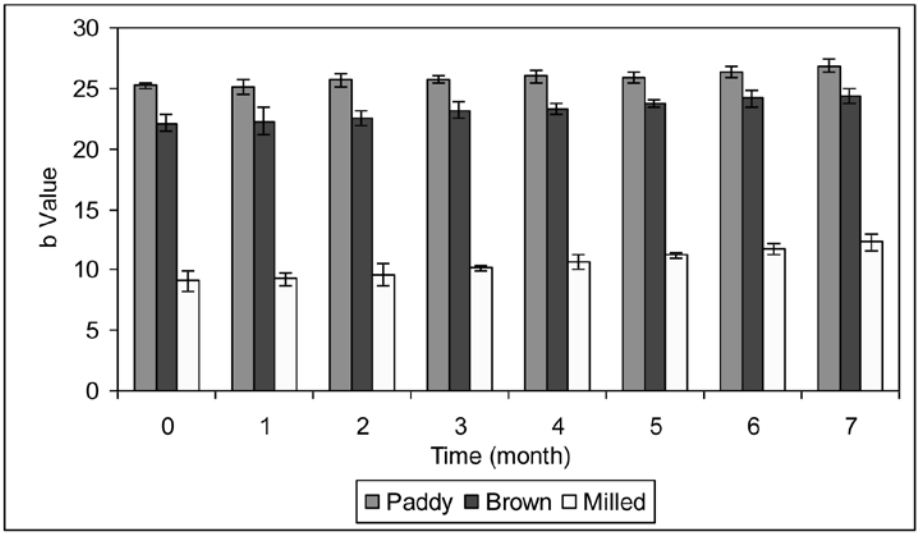

Figure 1. Effects of storage temperature and time on b value of the paddy, brown and milled rice during storage time and temperature at 25°C. (Data are means of at least two analyses with standard deviation. Error bar denotes ± 1 S.D. from the mean.)

Pasting and gelatinization properties

Stored rice caused alterations in the RVA properties from fresh harvest rice (Table 2). Peak viscosity, setback and final viscosity of stored rice increased 6%, 33% and 19%, respectively, comparing to fresh harvest rice.

Deprotein rice caused significant alterations in the RVA curves of fresh harvest rice (Table 2). Peak viscosity of fresh harvest rice decreased 24% in dealbumin rice, 44% in deglobulin rice, 3% in deprolamin rice, but increased 15%in deglutelin rice. Pasting temperature decreased in all materials be-

fore storage. Decreases in pasting temperature were observed in dealbumin rice, deglobulin rice, deglutelin rice and deprolamin rice. Pasting temperature of rice before storage decreased 15ºC in dealbumin rice, 2ºC in deglobulin rice, 14ºC in deglutelin rice and 16 ºC in deprolamin rice. Setback of deprotein rice decreased in all materials before storage except deglobulin rice. Decreases in setback were observed in dealbumin rice, deglutelin rice and deprolamin rice. Setback decreased 14% in dealbumin rice, 4% in deglutelin rice, 20% in deprolamin rice, but increased 6% in deglobulin rice. Final viscosity of fresh harvest rice decreased 14% in dealbumin rice, 15% in deglobulin rice, 39% in deglutelin rice, but increased 2% in deprolamin rice.

Table 1. Contents of protein isolates from fresh harvest rice and 7- month stored milled rice prepared by the traditional extraction (Osborne) method.

Protein	Fresh harvest rice		Stored rice	
	Protein content (g / 100 g)	Percentage (%)	Protein content (g / 100 g)	Percentage (%)
Albumin	0.2530 ± 0.004	4.7	0.1244 ± 0.0006	2.79
Globulin	1.0044 ± 0.003	18.78	0.7404 ± 0.0006	16.62
Glutelin	3.8560 ± 0.036	72.13	3.0896 ± 0.0305	69.35
Prolamin	0.2324 ± 0.001	4.34	0.3390 ± 0.0020	7.90
Total	5.3458	100	4.2934	100

Data are means of at least two analyses with standard deviation.

Table 2. Effect of albumin, globulin, glutelin and prolamin removal on RVA pasting properties of fresh harvest milled rice cv. Khaw Dok Mali 105.

Rice sample	Pasting Temp. (°C)	Peak Viscosity	Setback	Final viscosity
Native	93.05 ± 0.71	346.08 ± 0.59	69.58 ± 1.24	250.83 ± 0.29
Dealbumin	79.35 ± 0.60	264.83 ± 4.12	60.00 ± 0.65	215.00 ± 1.59
Deglobulin	91.25 ± 0.46	195.67 ± 1.83	73.75 ± 1.41	212.50 ± 1.77
Deglutelin	80.05 ± 0.07	398.42 ± 5.42	66.75 ± 1.41	152.75 ± 1.24
Deprolamin	77.75 ± 0.67	335.08 ± 3.00	56.00 ± 0.17	256.17 ± 1.06

Data are means of at least two analyses with standard deviation.

Table 3. Effect of albumin, globulin, glutelin and prolamin removal on RVA pasting properties of 7- month stored milled rice cv. Khaw Dok Mali105.

Rice sample	Pasting Temp.(°C)	Peak Viscosity	Setback	Final viscosity
Native	93.90 ± 0.53	367.08 ± 0.59	103.58 ±1.30	309.25 ± 0.88
Dealbumin	80.15 ± 0.53	183.58 ± 0.12	43.92 ± 0.82	122.00 ± 0.47
Deglobulin	93.90 ± 0.60	241.83 ± 4.06	98.08 ± 2.30	274.75 ±1.41
Deglutelin	80.10 ± 0.03	318.75 ± 6.66	58.92 ±1.24	156.83 ± 1.03
Deprolamin	87.25 ±1.06	453.17 ± 1.77	67.33 ± 0.53	282.58 ± 1.53

Data are means of at least two analyses with standard deviation.

Deprotein rice also caused significant alterations in the RVA curves of stored rice (Table 3). Peak viscosity of deprotein rice decreased in all deprotein rice samples after storage except deprolamin rice. Decreases in peak viscosity were observed dealbumin stored rice, deglobulin stored rice and deglutelin stored rice. Peak viscosity decreased 50% in dealbumin rice, 34% in deglobulin rice, 13% in deglutelin rice, but increased 23% in deprolamin rice. Pasting temperature decreased in all deprotein rice samples after storage except in deglobulin rice. Decreases in pasting temperature were observed in storage of dealbumin rice, deglutelin rice and deprolamin rice. Pasting temperature after storage decreased 15ºC in dealbumin rice, 15ºC in deglutelin rice and 7ºC in deprolamin rice. Set-back decreased in all materials after storage. Setback decreased 58% in dealbumin rice, 5% in deglobulin rice, 43% in deglutelin rice and 35% in deprolamin rice. Final viscosity of deprotein rice decreased in all deprotein rice samples after storage. Final viscosity decreased 61% in dealbumin rice, 11% in deglobulin rice, 49% in deglutelin rice and 9% in deprolamin rice.

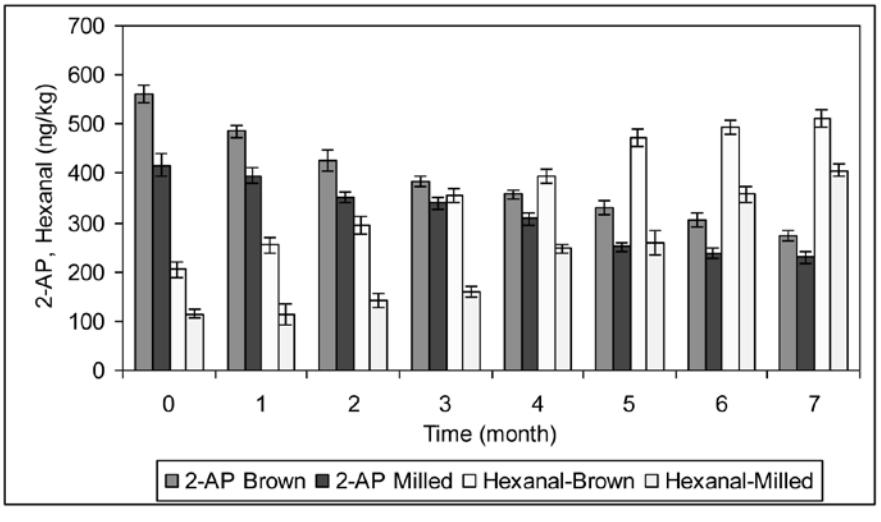

Figure 2. The 2-AP and hexanal contents of brown and milled rice during storage.(Data are means of at least two analyses with standard deviation. Error bar denotes ± 1 S.D. from the mean.)

The results demonstrate the instability of 2-AP in stored brown and milled rice (Figure 2). Browning volatiles detected, such as 2-AP, have been reported in cooked rice (Buttery et al., 1983). 2-AP was first reported as an important aroma component of aromatic rice and its occurrence in wheat bread crust, popcorn, and rice cake has also been reported (Buttery et al., 1988). Proline, a precursor of 2-AP, is also abundant in acha and its Strecker degradation product, l-pyrroline, has been shown to react readily with sugar degradation products to form 2-AP (Haq et al., 1995; Yoshikawa et al., 1965).

Hexanal was more increased in brown rice than in white rice. This pattern of increase suggested that the rate of oxidation on storage is increased due to the protective bran and hull. The chemical nature of the compound that increased (n-hexanal in particular, which was by far the major component of the rice volatiles) strongly suggests that lipid oxidation, probably accelerated by lipase and lipoxygenase activity in the rice.

Discussion and Conclusion

Deprotein caused significant alterations in the RVA curves of non stored and stored rices. Physicochemical properties of each type of deprotein rice altered in all rice samples and storage also affected the RVA behavior of rice. Other researchers have reported an increase in peak viscosity during storage of rice at lower temperatures (37, 20, and 3ºC) and 26ºC (Perdon et al., 1997; Sowbhagya and Bhattacharya, 2001).

The current study used ambient temperatures for 7 month durations of the storage process, which may have obscured the rise in peak viscosity.

Deprotein also decreased the rate of swelling of starch granules. As a consequence, higher temperatures were needed for starch swelling and viscosity development. Both fresh harvest rice and stored samples of dealbumin, deglutein, deprolamin and deprotein rice had decrease pasting temperature compared to non deprotein rice sample while only the aged deglobulin rice had not decrease pasting temperature caused by storage. Decrease pasting temperature is probably related that as deprotein materials, less time and energy were required to increase the viscosity of the gelatinized starch. However, decreased pasting slope shows that as deprotein materials, more time and energy were required to increase the viscosity of the gelatinized starch.

The cause of the altered starch granule swelling pattern in deprotein and non-deprotein of fresh harvest and stored rices is probably indicated the changes in the protein composition and starch. Peak viscosity was positively correlated to albumin, globulin and prolamin content and extractable proteins while only glutelin was negatively correlated in fresh harvest rice. Decreasing amounts proteins, albumin, globulin and prolamin, before storage and decreasing amounts, albumin, globulin and glutelin after storage, probably indicating that increased non-covalent associations within the starch granule may be partially responsible for the delayed and lower pasting properties of starch granules in grain. The difference in RVA properties between deprotein of fresh harvest and stored rice may be related to the storage time affected to the interaction of starch and protein compositions.

The decreased swelling behavior of starch may be related to the altered associations of amylose with amylopectin during annealing (Tester and Debon, 2000). Certain protein matrix probably becoming more closely associated with the starch granules during storage could explain the changes seen in the RVA behavior. During gelatinization, the protein matrix developed more crosslinks and acted as a barrier to water penetration,

hydration, and swelling of the starch granules. However, decrease amount of certain proteins might also decrease the water penetration, hydration, and swelling of the starch granules. The tight association of the protein matrix increased swelling of the granule thus increasing viscosity development. Altering the protein structure by using reducing agents has been shown to change the gelatinization character of starch (Hamaker and Griffin, 1993). Storage might increase the organization within the rice kernel, making it more susceptible to disintegration during mixing and increasing the ability of the starch to swell. In other rice storage studies, peak viscosity increased as storage increased (Zhou et al., 2003). These changes were dependent upon storage temperature and occurred more quickly at higher storage temperatures.

In this study, we determined 2-AP which is an important contributor to the character of fragrant rice flavour. The increase of this compound contributes to enhanced fragrance, as the flavour impact of this compound is critically related to its concentration. At relatively high level it contributes favourably, but at lower level it imparts an unpleasant note to rice. As the rice stored had a distinct aroma, it is probable that the level of 2-AP was rather low and that of other carbonyl compounds of off-flavours, such as hexanal, had exceeded their desirable limit.

In a complex flavour system as complex as that found for rice volatiles, it is not to be expected that the origins of all components could be explained by a single mechanism, and there were operating in parallel, the magnitude of the decreases of these decreases may not involve oxidation or enzymatic activity.

This study has demonstrated that the isolating rice protein fractions were very likely to have a correlation to the changes of pasting properties during storage. The rheological processes resulting in a paste during the formation of the peak will determine the behaviour of the paste/gel throughout breakdown and lift-off from the trough. Taken together, the results demonstrate that storage proteins influence viscosity curves probably through binding water, which cause the concentration of the dispersed and viscous phases of gelatinised starch. Removal of storage proteins affected the peak height. Interpreting the viscosity curve, proteins may affect the amount of water the rice absorbs in pasting, and the availability of water early in pasting will determine the hydration of the protein and the concentration of the dispersed and viscous phases of the starch.

RVA was performed on flour of rice cv. Khaw Dawk Mali 105 with the individual storage protein fraction removed. Deprotein rice flour sample was found to cause a significant change in RVA behavior the starch gel. Recent studies suggest that protein can play a role in determining the pasting and textural properties of rice. Lim et al (1999). reported that reducing the protein content in rice flour increases its peak viscosity. This was confirmed by Tan and Corke (Tan and Corke, 2002) who proposed that protein content is negatively correlated to peak viscosity and hot paste viscosity. Furthermore, Lyon et al (2000) found that protein content was negatively correlated to adhesiveness of cooked rice. Some preliminary studies have suggested that glutelin and the 60 kDa starch granule bound starch synthase protein are related to adhesiveness and other textural characteristics (Hamaker and Griffin, 1993). The present study suggests that storage proteins can significantly affect viscosity of rice pastes of rice flour gels. The procedure used for the extraction of storage proteins from rice flour produced a protein that the changes in the pasting and viscosity properties of rice starch (Tables 2 and 3) Furthermore, the storage protein extraction process did not significantly altered the RVA profile of the rice flour samples, suggesting that the

properties of storage protein and other rice components had not been modified significantly by extraction to cause a significant change in their pasting behaviour. This indicates that the observed changes in the pasting properties of rice flour following the removal of storage protein was due to the absence of the protein in the samples. The individual storage protein removed from flour was a complicated system consisting of starch, other storage proteins and other rice components. It is generally accepted that the increase in viscosity that occurs during heating of starch suspension is mainly due to the swelling of the starch granules with lesser contributions from the solubilisation of amylose and hydration of protein (Sandhya Rani and Bhattacharya, 1995). The gel formed at the end of the RVA cooling cycle is essentially a three-dimensional network of intertwined amylose molecules incorporating dispersed swollen and ruptured starch granules.

The decreased final viscosity of samples without individual protein fraction suggests that the three dimensional network is weakened by the absence of protein in the matrix. Interestingly, Chrastil (1990) found that the adhesiveness of rice increases with the amount of glutelin it contains. The rice proteins were able to form stable gels at a low concentrations and the presence of disulphide bonds may have contributed to this effect. The major protein (glutelin) fraction possesses good gelling properties. Proteins affect the amount of water the rice absorbs in gelatinization, and the availability of water early in pasting will determine the hydration of the protein and the concentration of the dispersed and viscous phases of the starch, which will determine the viscosity of the gelatinized rice starch. Higher ratios of insoluble protein together with less disulphide bonding could show less viscosity. Alternatively it has been suggested that protein encircles the starch granules, inhibiting swelling, (Tamaki et al., 1989) but this is unlikely since proteins are in either spherical or crystalline bodies (Hoseney, 1994). Furthermore, if proteins encircle the starch granules in a way that inhibits swelling, presumably the higher the protein content, the thicker would be the encircling layer causing a slower rate of pasting, suggesting that a suite of proteins with less potential to form a disulphide bonded network explains the lower peak.

On the effect of individual storage protein on viscosity of stored rice, presumably the processes leading to the peak result in a gel or paste that will behave in a particular way during breakdown). Proteins contributed to: the height of the peak the rate of pasting, the amount of breakdown in the rice starch, and to final viscosity. In each deprotein rice sample, individual storage protein contributed differently to each part of the curve viscosity, suggesting for each storage protein either a different amount, suite or molecular weight distribution (Chrastil, 1994) of proteins, or different interactions with starch. The factors governing the formation of the peak would include swelling of the starch granules to form the dispersed phase (Nguyen et al., 1998), hydration of proteins binding a disproportionate amount of water11 and solubilisation of amylose to form the viscous phase (Chrastil, 1990). Without proteins the rate of pasting should reflect only the swelling of the starch. The difference in the rate of pasting indicates the contribution of protein to that rate. The storage proteins should be slightly below that of starch (Haq et al., 1995), which could explain why proteins affect the rate of pasting early in the peak. The difference between the viscosity pattern is unique to each deprotein rice sample, perhaps defining the role of proteins in pasting, and in the first processes of cooking. Removing proteins decreased the lift-off from the trough to the final viscosity. Perhaps that was due to biological and rheological events preceding the trough. Since proteins account for only about 8% of

the flour removing proteins will increase the amount of available water thus diluting the dispersed and viscous phases of the starch.

Without proteins, the amount of breakdown was the same or greater relative to peak height, suggesting that the lower viscosity did not protect the paste from shear. The lift-off to final viscosity describes the aggregation of amylose molecules into a gel (Tsai et al., 1997). If removing proteins decreased the concentration of the viscous phase and increased its susceptibility to shear, then the dilute, sheared viscous phase would be less able to form a viscous gel (Nguyen et al., 1998). The effects of individual storage protein fraction on viscosity may mean that the viscosity of rice is determined, at least in part, by the relative proportions of the protein fractions in rice. It may also mean that it might be possible to develop rice with desired levels of viscosity by storage condition with particular proportions of storage proteins.

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