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How Do Endosperm Proteins Affect the Pasting and Texture of Rice? – A Study of the Physiochemical Properties of Rice Using a Model System

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Abstract

Protein is the second most abundant component of rice, but its influence on the physiochemical properties of rice is not clear. Albumin, globulin, prolamin and glutelin were added separately to rice starch at pre-determined concentrations, and the pasting and textural properties of the resultant mixtures were analysed by the Rapid ViscoAnalyser (RVA) and the TA XT2 Textual Analyser. Addition of albumin to rice starch caused significant ($P < 0.01$) increases in all the pasting parameters measured and the textural property of gel hardness, but significant decreases in gel adhesiveness. In contrast, addition of globulin caused significant ($P < 0.01$) decreases in all the pasting parameters and gel adhesiveness, but increases in hardness. For the two storage proteins, increasing the concentration of prolamin in starch resulted in decreases in pasting temperature, peak and final viscosities, gel hardness and adhesiveness, but increases in breakdown viscosity. In contrast, increasing the concentration of glutelin resulted in decreases in peak, breakdown and final viscosities, but increases in pasting temperature, gel hardness and adhesiveness. A model rice system was created by adding to rice starch albumin, globulin, prolamin and glutelin in different ratios and the pasting and textural properties of the resultant model rice flour were compared with those of rice starch control. The results show that the physiochemical properties of the model rice system are broadly comparable with native rice of similar composition. Our results indicate that such a model system can be a useful tool for studying the physiochemical properties of rice.

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Introduction

Pasting and texture are two of the most important physiochemical properties of rice as they are closely associated with the processing and sensory characteristics of rice (Baxter et al. 2004). Despite its importance, it is not fully understood how rice components affect those properties. Much of the research in this area has focused on the influence of starch on those properties with considerable success (Kokini et al., 1992; Vandeputte et al., 2003). However, some studies have shown that rice cultivars with similar starch content and composition exhibit rather different pasting and textural characteristics (Champagne et al., 1999), suggesting that other components may also play a role.

Protein is the second most abundant component of rice, accounting for about 9 % of the dry weight of milled rice (Kennedy and Burlingame, 2003), but its influence on the physiochemical properties of rice is not clear. Lim et al. (1999) reported that a reduction in the protein content of rice flour result in an increase in its peak viscosity. This was confirmed by Tan and Corke (2002), who showed that protein content is negatively correlated to peak viscosity and hot paste viscosity. Furthermore, Lyon et al. (2000) found that protein content is negatively correlated to the adhesiveness of cooked rice. While these studies have established a link between total protein and the physical properties of rice, the role played by individual protein classes (albumin, globulin, prolamin and glutelin) in determining the pasting and textural properties of rice is not well understood.

We have studied the effect of endosperm proteins on the processing and sensory properties of rice and found that individual protein fractions can affect rice pasting and texture in very different, and sometimes opposing manner. This makes it difficult to ascertain the role played by individual proteins in native rice as the effect of the proteins may cancel each other out.

To overcome these difficulties, we have developed a model rice system that enables us to determine the effect of both individual proteins and protein-protein interactions on the physical properties of rice. The concept of model systems, where protein of a defined concentration is added to a standard starch, has been explored in other cereals such as wheat (Fido et al., 1997). However, using such a system for studying rice proteins has not been reported. The rice model system that we have adopted allows complex studies to be performed in a matrix of more defined composition than native rice flour and therefore the interactions between the protein fractions and other rice components can be determined. Here we report some initial results of the study.

Materials and Methods

Materials

Three cultivars of rice (Amaroo, Opus and Langi) were used in this study. The varieties were grown at the Yanco Agricultural Institute, Yanco, NSW, Australia, during the 2001/2002 growing season. Rice grains with an average of 12 % moisture were dehulled (THU35A Test Husker, Satake) and then milled (McGill No. 2 Mill) for 60 s. Broken grains were separated from whole grains by weight differentials and only whole grains were used for this study. Milled grains were ground to pass through a 0.12 mm screen (Retsch model, Zm100). Rice starch was obtained from Sigma Aldrich Pty Ltd (Product No. S 7260).

Defatting of rice flour

Flour samples were defatted before protein extraction in the experiments involving the addition of protein to commercial rice starch in order to minimise lipid contamination in the protein extracts. Defatting was carried out by adding 14 volumes of a 1:1 ethyl ether/methanol mixture to rice flour samples, and the resultant slurries were mixed thoroughly, allowed to stand for 1 h and centrifuged at 10,000*g* for 10 min (Chrastil and Zarins, 1994). The defatting procedure was repeated a total of three times to maximise removal of the lipid components.

Extraction of proteins

Rice flour was extracted sequentially with water, 1.5 M NaCl, 100% propan-2-ol and 0.1 M NaOH to extract albumin, globulin, prolamin and glutelin respectively. For each extraction, the suspensions were mixed thoroughly, allowed to stand for 30 minutes, mixed thoroughly again and centrifuged at 10,000 g for 10 min at 15 °C. The extraction procedure was performed a total of three times for each solvent and the supernatants pooled. The globulin, prolamin and glutelin extracts were dialysed against de-ionised water overnight at room temperature. The protein extracts were used for preparing model rice flour mixtures with defined composition as described below. Protein concentrations for each extract were determined by the Lowry method using the Biorad DC protein assay kit (Catalogue No. 500-0111). Standard curves used to determine protein concentration were created for each of the different solvents using lyophilised rice albumin, globulin, prolamin and glutelin dissolved in the respective solvent.

Model rice flour mixture with defined composition

Protein extracts of albumin, globulin, prolamin or glutelin, obtained as described above, were used for preparing rice flour mixtures with defined composition. For experiments on the effect of individual proteins on the physical properties of rice starch, individual protein extracts were added to rice starch at pre-determined amounts to give flour mixtures containing either up to 20 mg/g starch of albumin, 45 mg/g starch of globulin, 60 mg/g starch of prolamin or 100 mg/g of glutelin. In separate experiments, a model rice system was created with similar protein compositions to those of native rice. Protein was added in a ratio of 5:20:30:50 for albumin, globulin, prolamin and glutelin respectively to give the standard model rice mixture. Four separate rice models were then created using the same protein composition of the standard model. Each model, however, was enriched with four times the standard amount of either albumin, globulin, prolamin or glutelin. Furthermore, two more models were created where each protein fraction was added using the same relative proportion as that in the standard formulation but at twice and fours times the concentration. The pasting and textural properties of each rice flour mixture were analysed.

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RVA analysis

Pasting properties of rice starch and model flour mixtures, created as described above, were determined using a Rapid-Visco Analyser (Newport Scientific model 3D, Warriewood, Australia) following the AACC Approved Method 61-02 (2000), modified by extending the cooling time by 5 min and holding at 50 ^{o}C for 5 min to ensure that maximum peak viscosity was obtained. Each RVA canister contained 3 g of starch or flour and was made up to 28 g with de-ionised water or extracted protein in de-ionised water. Peak viscosity, hot paste viscosity, final viscosity, breakdown (peak viscosity - hot paste viscosity) and setback (final viscosity - peak viscosity) were recorded. Each analysis was performed at least twice.

Textural analysis

Textural properties of the rice flour gels formed after RVA analyses were determined with a TA XT2 textural analyser (Stable Microsystems, Surrey, Great Britain). Gels were sealed in the canisters with paraffin film to prevent moisture loss and were left overnight at 4°C to allow even retrogradation. Analyses were carried out using a standard two-cycle program (TPA procedure) with a 10-mm cylindrical ebonite probe, which was programmed to move downwards for a distance of 48 mm at a speed of 2 mm/sec. From the force-time curve obtained, textural parameters of hardness (height of the force peak on cycle 1, g), cohesiveness (ratio of the positive force areas under the first and second cycles), adhesiveness (negative force area of the first cycle, -gs) and gumminess (hardness × cohesiveness, g) were computed using the Texture Expert software supplied with the instrument.

2.7. Statistical analysis

Data obtained were analysed by analysis of variance (ANOVA) following the procedure described by Miller and Miller (1993) using SPSS for Windows^{TM} version 11.0. Differences of means were reported at the 5 % significance level.

Results

Effect of adding individual proteins on the physical properties of rice starch

Addition of albumin was found to cause a significant ($P < 0.01$) increase in all of the pasting parameters measured as well as the textural property of gel hardness, but a significant decrease in gel adhesiveness (Table 1). In contrast, addition of globulin to rice starch caused a significant ($P < 0.01$) decrease in all the pasting parameters and gel adhesiveness, but an increase in gel hardness. For the two storage proteins, increasing the concentration of prolamin in rice starch resulted in decreases in the pasting properties of pasting temperature, peak viscosity and final viscosity as well as the textural properties of gel hardness and adhesiveness, but increases in breakdown viscosity. In contrast, an increase in the concentration of glutelin resulted in a decrease in peak viscosity, breakdown viscosity and final viscosity, but an increase in pasting temperature, gel hardness and adhesiveness.

Effect of different proteins on the physical properties of rice in a model system

In order to determine the effect of protein composition on pasting and textural properties, a model rice system is created with similar protein compositions to those of native rice. Protein was added in a ratio of 5:20:30:50 for albumin, globulin, prolamin and glutelin respectively (referred to as the standard formulatation in proceeding text). The pasting and textural properties of the resulting mixtures were compared to those of a starch control (Table 2). In addition, to further investigate the effect of each individual fraction on rice pasting properties, four separate rice models were created using the protein composition of the standard model. Each model however, was enriched with four times the standard amount of either albumin, globulin, prolamin or glutelin. The determined pasting properties were compared to the properties of the starch control and the standard formulation (Table 2).

Addition of protein to rice starch in a ratio of 5:20:30:50 for albumin, globulin, prolamin and glutelin, respectively, resulted in significant ($P<0.05$) increases in pasting temperature and gel hardness, but significant decreases in gel adhesiveness (Table 2). Interestingly, however, the addition of proteins did not significantly change peak viscosity.

Effect of enrichment of individual proteins on the physical properties of the model rice flour

The formulation enriched with albumin had a significantly higher pasting temperature and peak viscosity and final viscosity than both the starch control and the standard formulation. Enrichment of albumin by four times also resulted in further decreases in gel adhesiveness. In contrast, albumin enrichment resulted in a decrease in gel hardness compared to the standard formulation.

The model rice flour mixture enriched with globulin had a significantly ($P<0.05$) lower pasting temperature than both the starch control and the standard formulation. The globulin enriched flour also had a significantly ($P<0.05$) lower peak viscosity compared to the standard formulation. Globulin enrichment, however, did not result in any further significant changes in gel hardness or adhesiveness.

Enrichment of prolamin by four times the standard formulation resulted in a significantly ($P<0.05$) lower pasting temperature when compared to both the starch control and the standard formulation. A reduction in peak viscosity was observed in the prolamin enriched flour when compared to the standard control and starch control. This reduction, however, was not significant ($P>0.05$) in either case. On the other hand, prolamin enrichment resulted in significant decreases in both gel hardness and adhesiveness.

The model rice flour enriched with glutelin had significantly ($P<0.05$) higher pasting temperature, but significantly lower peak viscosity compared to both the starch control and the standard formulation. Glutelin enrichment also caused significant increases in both gel hardness and adhesiveness.

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Effect of higher protein concentrations on the physical properties of the model rice flour

To further understand the interactions between the different protein fractions and starch, each protein fraction was added using the same relative proportions that was used in the standard formulation but at an increased level (2 or 4 times, henceforth known as standard x 2 and standard x 4 respectively) (Table 2). Increasing the protein content by a factor of two or four times resulted in significant ($p<0.05$) increases in pasting temperature and breakdown viscosity. The peak viscosity also increased in the formulations with higher protein content, however the difference was not significant ($P>0.05$). Increasing the protein content by a factor of two or four times, without altering the protein ratios of the standard formulation, resulted in significant ($p<0.05$) changes in the textural properties. The hardness value in the standard x 2 and x 4 formulations were significantly higher than both the starch control and the standard formulation. A significant ($P<0.05$) reduction in adhesiveness was also observed.

Discussion

In a previous communication (Baxter et al., 2004), we have shown that the storage protein, prolamin, can significantly affect both the pasting and textural properties of rice flour. Charastil (1992) reported that the other storage protein, glutelin, can affect the textural properties, and that the gel adhesiveness of rice flour increase with the amount of glutelin it contains. The currently study has confirmed those trends. Furthermore, we have shown that glutelin can also have a significant influence on rice pasting properties including pasting temperature and peak viscosity, therefore demonstrating that the protein can affect the pasting as well as textural properties of rice. Interestingly, the effects of glutelin and prolamin on the physical properties of rice flour often exhibited opposing trends. For example, an increase in the concentration of prolamin resulted in a decrease in pasting temperature, gel hardness and adhesiveness, but an increase breakdown viscosity, whereas the exact opposite trends were observed with an increase in the concentration glutelin. We have previously reported (Baxter et al. 2004) the contrasting effects of prolamin and glutelin on textural properties of rice. The current study has shown that similar phenomenon also occurs in some of the pasting characteristics of rice. As glutelin and prolamin are the two predominant protein fractions in rice, accounting for more than 80% of the total endosperm protein content, the contrasting effects of the two proteins on some of the pasting and textural properties may mean that those characteristics are determined, at least in part, by the relative proportions of the two proteins in rice.

Studies conducted in our laboratory have also shown (data not shown) that the concentration of glutelin and prolamin can vary considerably among different rice cultivars. Furthermore, even the same cultivar can show remarkable variations in the relative proportion of the two proteins when grown under different agronomical conditions such as the timing of nitrogen fertilisation. Specifically, we have observed (data not shown) that while applying nitrogen fertilisers either pre-flood or at panicle initiation resulted in significant increases in the total protein content of rice, the increase was mainly attributable to increases in the concentration of glutelin with the former practice. In contrast, the increase in the concentration of prolamin accounted for the majority of the observed increase in endosperm protein when nitrogen was applied at the panicle initiation stage. These findings mean that it might be possible to

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produce rice with desired levels of certain pasting and textural characteristics through targeted breeding programs and agronomical practice.

The two metabolic proteins, albumin and globulin, have also been shown to affect the pasting and textural properties of rice starch. Interestingly, the effects of the two proteins on rice physical properties also exhibited opposing trends, especially regarding their effect on rice pasting properties. These contracting effects of the different proteins on the physical properties of rice could mean that the observed pasting and textural characteristics of native flour is, notwithstanding the contribution of starch and other components, probably a result of their relative proportions in rice. This is clearly seen in the results obtained from model rice flours with defined compositions. For example, when the model rice flour with the standard formulation is compared with pure starch, it is observed that the differences in pasting temperature and peak viscosity between the two samples were relatively small. In the case of peak viscosity, the difference was statistically insignificant ($P>0.05$). This is probably a reflection of the observation that albumin and glutelin had the effect of causing increases in pasting temperature but decreases in peak viscosity, whereas globulin and prolamin had exactly the opposite effect on the two pasting parameters. Thus, the effects of the four different proteins on pasting temperature and peak viscosity have essentially concealed each other out. On the other hand, the model rice flour had significantly higher gel hardness but lower gel adhesiveness compared with the starch control. This is likely a reflection of the observation that albumin, globulin and glutelin all had the effect of causing increases in gel hardness, whereas only prolamin had the opposite effect. On the other hand, albumin, globulin and prolamin all had the effect of increasing gel adhesiveness while only glutelin had the opposite effect.

When the proportions of the different proteins were varied, their effects on rice physical properties became much more complicated and difficult to predict. In order to make such predications, more detailed and quantitative studies on the effect of individual proteins on the pasting and textural properties of rice are required and such studies are being carried out in our laboratory.

In conclusion, the current study has shown that all the four major classes of rice protein, namely albumin, globulin, prolamin and glutelin, can significantly affect both the pasting and textural properties of rice and the effects of the different proteins often exhibit opposing trends. The model rice system that we have developed can be a useful tool for studying the effect of both individual proteins and protein-protein interactions on the physical properties of rice.

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Table 1. Effect of individual endosperm proteins on the RVA pasting and TA XT2 textural properties of rice starch

Increasing the concentration of	Resultant change in physical properties of rice					
	Pasting properties				Textural properties	
	Pasting Temperature (°C)	Peak viscosity (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Gel hardness (g)	Gel adhesiveness (-gs)
Albumin	⇧	⇧	⇧	⇧	⇧	⇩
Globulin	⇩	⇩	⇩	⇩	⇧	⇩
Prolamin	⇩	⇩	⇧	⇩	⇩	⇩
Glutelin	⇧	⇩	⇩	⇩	⇧	⇧

Table 2. Effect of endosperm proteins on the pasting and textural properties of rice flour in a model system with defined composition

Protein concentration (mg/g starch)				Pasting properties[a,b]		Textural properties[a,b]	
Albumin	Globulin	Prolamin	Glutelin	Pasting temperature (°C)	Peak viscosity (RVU)	Gel hardness (g)	Gel adhesiveness (-gs)
0	0	0	0	78 ± 0.07[C]	244 ± 2.4[BC]	129 ± 2.7[B]	178 ± 2.4[D]
5	20	30	50	79 ± 0.04[D]	245 ± 1.3[C]	185 ± 2.5[D]	159 ± 1.6[C]
20	20	30	50	80 ± 0.09[E]	284 ± 3.1[D]	161 ± 2.1[C]	151 ± 2.1[C]
5	80	30	50	76 ± 0.05[B]	235 ± 2.7[B]	188 ± 1.8[D]	157 ± 1.8[C]
5	20	120	50	74 ± 0.08[A]	240 ± 3.1[BC]	94 ± 2.1[A]	68 ± 3.4[A]
5	20	30	200	84 ± 0.06[G]	161 ± 2.7[A]	206 ± 4.8[E]	219 ± 1.8[E]
10	40	60	100	80 ±0.07[E]	247 ± 2.5[BC]	206 ± 4.2[E]	91 ± 2.4[B]
20	80	120	200	80 ±0.05[F]	251 ± 3.2[C]	216 ± 2.5[E]	85 ± 1.9[B]

[a]Each value represents the mean of three measurements.
[b]Data containing the same superscript letter, in the same column, are not significantly different at the 5% level.