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Viscosity of Rice Flour: A Rheological and Biological Study

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Rice breeders frequently use rapid visco analysis (RVA) as in index of rice quality. Potentially, viscosity curves could also be used to predict the sensory properties of a sample of rice or the processing properties of rice when used as an ingredient. The aim of this study is to determine the contribution of the main components of rice flour−starch and protein polymers and lipids−to the viscosity curve, accounting for biological and rheological contributions, and interactions with water. By deconstructing the rice flour, resistance to shear is generally the primary factor that affects rheological processes in the RVA, often masking the physical processes of cooking. Thus, higher concentrations of water reveal more about the true biological and physical processes of the transition from a powder to paste. Proteins contribute to peak height, offset thixotropy, and contribute to the final viscosity. Starch−lipid complexes can mask differences in the molecular structures of amylose and amylopectin, and removing lipids alters the structure of the paste significantly, which consequently alters viscosity curves.

KEYWORDS: Viscosity; rice; starch; proteins; lipids

INTRODUCTION

Viscosity curves, by rapid visco analysis (RVA), are one of the most useful tools for rapidly and reproducibly assigning rices to different quality classes, but are probably underutilized because the biological and rheological contributions of starch and protein polymers and lipids to forming the curve are unknown. The formation of a viscosity curve involves a transition from a powder of semicrystalline polymers and complexed lipids to a paste of gelatinized and denatured polymers and lipids. The onset of viscosity begins in excess water at about 65 °C when amylopectin crystals begin to melt (*1*) and proteins hydrate (*2*, *3*). As the sample is heated further, viscosity rapidly increases as starch granules swell, amylose leaches (*4*, *5*), some amylose complexes with lipids (*6*), and proteins presumably denature. The peak viscosity occurs when swelling and shear are balanced. The composition of the paste at the peak would influence the next stage, from peak to trough viscosity (breakdown), during which the temperature is constant. During breakdown, the paste displays thixotropic behavior, either due to alignment or mechanical breakdown of polymers. The third region of the curve, from the trough to final viscosity (lift-off), occurs as the system cools to 50 °C. The composition of the paste at the trough also influences lift-off (*7*), during which amylose molecules aggregate into a network, embedding remnants of starch granules, with proteins likely to contribute also.

Viscosity curves are the most useful tool available for rapidly and reproducibly assessing cooking quality of rice, so understanding how the curve is formed is the first step in determining how the parameters of the curve can be translated into sensory or processing attributes. For many years, the setback, defined by subtracting the peak viscosity from the final viscosity (*8*), has been related to the firmness and the amylose content of the rice (*8*), and breakdown, defined as subtracting the viscosity at the trough from the viscosity at the peak (*8*), has too (*9*). Stickiness has been correlated with final viscosity, and cohesiveness of mass has been correlated to setback (*9*), but in that study, setback was calculated by subtracting the viscosity at the trough from the final viscosity (*9*). Another sensory property, cohesiveness of mass, is defined as the maximum degree to which a sample of cooked rice holds together during chewing, and this correlated weakly with breakdown (*9*). Further, it was concluded that viscosity curves were not able to predict or model sensory qualities with high accuracy (*9*). Intuitively, this is difficult to accept since viscosity curves generated by RVA are produced by mimicking the process of cooking; viscosity is measured as resistance to stirring as the slurry of flour and water is heated. The processes and interactions that occur during heating and stirring are likely to depend on the physical characteristics of the different components of the flour, as do the properties of a cooked grain of rice. It seems therefore that characteristics of the curve could be used either as a direct or indirect measure of the properties of cooked rice.

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The objective of this study is to determine the contributions, individually and collectively, of amylose, amylopectin, proteins, lipids, and water toward the formation of a viscosity curve.

MATERIALS AND METHODS

Contribution of Starch, Proteins, Lipids, and Water to the Viscosity Curve. Three pairs of rice cultivars, *Oryza sativa*, were chosen, on the basis of similar amylose content. Two contained no amylose, two contained 18% amylose, and two contained 24% amylose. The attributes of individual cultivars is beyond the scope of this work, but pairs are used to illustrate that factors other than amylose content are at play.

All cultivars, except one with 24% amylose, were grown in the 1999/2000 season on clay loam at the Research farm of Yanco Agricultural Institute, Yanco. They were fertilized just prior to flooding with urea at the rate of 150 kg N ha^{-1}. The rice was harvested at maturity, when the grains were at 14% moisture. When the rice reached 12% moisture, paddy rice (150 g) was dehulled (THU35A Test Husker, Satake) and then milled (McGill No. 2 Mill) for 60 s. The broken grains were separated from whole grains, and the broken grains were discarded. All RVA data was collected within 3 months of milling. The variety not grown in Australia was stored at 4 °C from the time of purchase until the time of use.

Milled rice grains (∼50 g) were ground to pass through a 0.5 mm screen (Cyclotec 1093 sample mill, Tecator, Hoganas, Sweden). Flour (2, 3, or 4 g) was weighed into an aluminum canister, to which 25 g distilled water was added. Viscosity was measured by RVA (Newport Scientific model 3D) using the profile outlined by Approved Method 61−02 (*10*). Two replicates of each sample were run.

Protein content was determined in duplicate by Leco analysis and was about 8% for the two waxy cultivars and ranged from 5 to 8.5% for the four nonwaxy cultivars. Proteins were removed from the flour, as previously described (*11*), to determine their effect on viscosity curves. Briefly, Tricine-protease buffer (25 g, 500 mM, pH 7.5, containing 10 U Protease (Sigma)) was added to the flour (either 2, 3, or 4 g) in the RVA canister instead of water. The RVA profile was run as previously described (*11*). Flour from all six varieties was treated with the Tricine-protease buffer as described above. Viscosity was measured as described earlier on flour or defatted flour from all six cultivars. Each sample was prepared and run in duplicate. Every effort was made to ensure that the protease did not contain enzymes that could degrade any other component of the rice flour, but trace amounts of other enzymes cannot be ruled out.

Lipids were removed from the flour of all six cultivars by refluxing for 20 h in 85% methanol (*12*). The defatted flour was dried overnight and again passed through a sieve. Viscosity of the treated flours was determined by removing proteins from the defatted flours (2, 3, or 4 g) using the protease treatment and extended RVA profile as described above. Following defatting, amylose content and protein content of the flours were not significantly different to their contents in whole flour. Each sample was run in duplicate.

Effect of Treatment on Damaged Starch and Amylase Activity. In order to test whether the treatments to remove proteins and lipids damaged the starch or increased the susceptibility of the starch to endogenous amylase activity, the flour and treated flours were assayed for damaged starch and for endogenous amylase activity. The assays were conducted using a kit (Megazyme) and the Approved Method 76-31 (*13*). In brief, damaged starch was measured by incubating the flour or treated flour with fungal α-amylase for 10 min at 40 °C. The reaction was terminated, then the mixture was centrifuged. Starch in the supernatant (accessible to α-amylase) was measured by digesting it to glucose, oxidizing the glucose, and measuring absorbance of the products at 510 nm. Endogenous amylase activity was measured by following the above procedure, but omitting the fungal α-amylase.

Effect of Treatment on Amount of Hot-Water-Soluble Amylose. The amount of amylose leached from flours of the different treatments was measured by weighing 500 mg of flour or treated flour into an aluminum RVA canister to which 12.5 g of distilled water was added. The slurry was heated and stirred using the RVA profile defined by the Approved Method 61-02 (*10*). The viscosity run was terminated when the temperature reached 95 °C. The contents of the canister were immediately transferred to a centrifuge tube and centrifuged at 3000*g*, and an aliquot of the supernatant solution was injected immediately into a GPC column. Chromatography was performed using a Waters 2690 Alliance, a Waters 2410 Refractive Index detector, and a Waters Ultrahydrogel 500 gel permeation column. Mobile phase was 0.05 M ammonium acetate at a flow rate of 0.5 mL min^{-1}, column temperature 60 °C, and the run time was 40 min. The program Millennium was used to control the pump, and to acquire and process data.

Table 1. Peak (P), Trough (T), and Final (F) Viscosities (RVU) of Curves in **Figure 1**

variety (%) and treatment	2 g			3 g			4 g		
	P	T	F	P	T	F	P	T	F
full flour									
0	250	50	61	151	85	108	250	115	152
0	65	61	75	178	118	150	294	163	230
18	70	48	96	242	138	239	495	227	374
18	77	55	106	279	154	253	546	248	396
25	49	34	71	209	137	263	454	244	459
25	30	20	39	195	125	283	434	272	566
flour without proteins									
0	11	8	11	18	11	17	29	15	23
0	46	27	35	88	48	63	136	73	102
18	46	32	65	159	80	140	329	132	212
18	46	32	63	160	83	139	319	129	201
25	38	28	67	138	87	176	329	174	329
25	36	28	75	137	84	191	319	180	361
flour without lipids or proteins									
0	75	46	54	152	85	106	239	119	169
0	64	44	52	131	85	106	207	124	176
18	38	35	56	148	104	164	376	178	299
18	42	37	57	158	100	155	387	167	281
25	31	29	46	119	96	156	298	185	318
25	32	31	44	131	107	166	339	194	340

RESULTS

To understand the interactions between the major components of rice (proteins, lipids, starch, and water) and their contribution to viscosity, concentration was varied and the flour was deconstructed. The change in viscosity by removing a component allowed the actual effect of the component to be recognized. Two replicates of each sample were run, but data from only one are shown since replicates replicated very well. **Figure 1** shows the curves and **Table 1** reports the peak viscosity, trough viscosity, and final viscosity for each sample. Breakdown is the difference between peak and trough viscosity, setback is the difference between final and peak viscosity, and lift-off is the difference between trough and final viscosity.

Contribution of Water (F). As the concentration of water was decreased, the effects on viscosity were similar for each of the treatments (**Figure 1**; **Table 1**). The concentration of water affected the time and the temperature of the onset of pasting slightly, the gradient of the pasting curve increased greatly as the concentration of water decreased (**Figure 1**), and breakdown increased, lift-off decreased and therefore setback is more negative. As the concentration of water was decreased, the peak height of the waxy varieties increased linearly, but that of the nonwaxy varieties increased exponentially.

Contribution of Proteins (F−p). Figure 1d−f shows viscosity curves of flour after removal of the proteins. The peak, trough and final viscosities are lower for each sample (**Figure 1**; **Table 1**). Nonwaxy pairs become indistinguishable and the waxy pair separate further. Moreover, for the nonwaxy varieties onset times coincide, but relative to flour, are delayed. The gradient from onset to peak is steep, the amount of breakdown

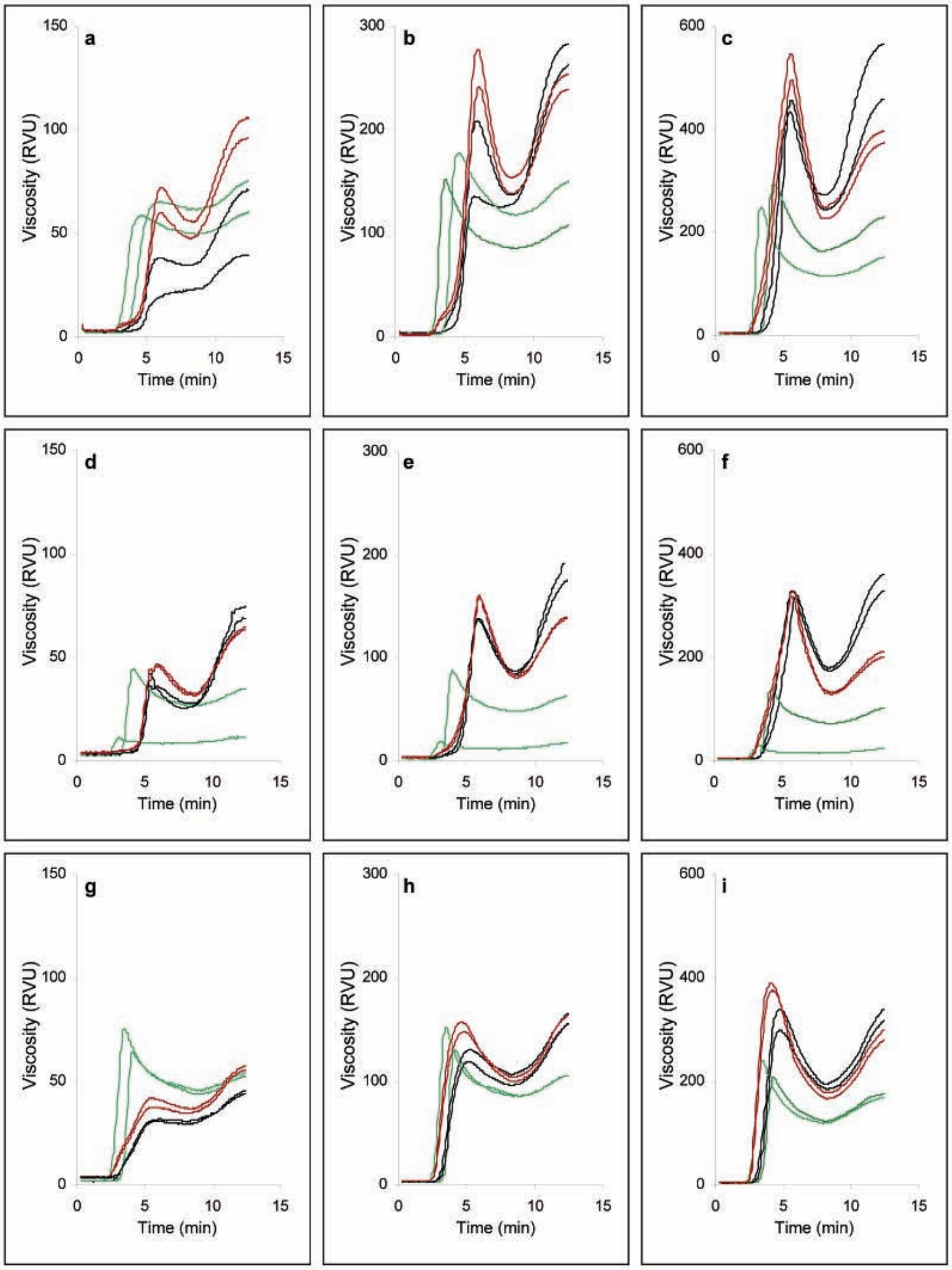

Figure 1. Viscosity curves of six rice varieties at three concentrations of water (8, 12, or 16%) of full flour (a–c), flour with proteins removed (d–f), and flour with proteins and lipids removed (g–i): two varieties are waxy and contain no amylose (green), two contain 18% amylose (red), and two contain 24% amylose. Note: The scale on the *y*-axis is to 150, 300, or 600 RVU for the 8, 12, and 16% water treatments, respectively.

relative to the peak height is proportionally more without proteins than in flour, and final viscosity is lower than in flour. Waxy varieties undergo more breakdown and similar lift-off, with one producing negligible viscosity.

Contribution of Lipids and Proteins (F−l−p). Figure 1g−i shows the effect that the removal of lipids and proteins has on viscosity. Relative to flour, nonwaxy varieties exhibit a shallow gradient from onset to peak producing a lower peak. Again relative to flour, breakdown is negligible and lift-off is lower. Nonwaxy varieties also become closer, but are not superimposed, and have about half the peak viscosity of the waxy pair. Compared to flour, the order of peak viscosity is switched for the 0 and 24% pair with that difference becoming clearer as the concentration of water decreases.

Hot-Water-Soluble Amylose (HWSA). The amount of hot-water-soluble amylose (HWSA) that was leached from flour or treated flour from each nonwaxy variety is shown in **Figure 2**. The varieties of 24% amylose consistently produce more HWSA than the varieties of 18% amylose. When proteins were removed from the flour, the amount of HWSA was less (**Figure 2**), but when lipids and proteins were removed, more HWSA was measured than from flour.

Damaged Starch. Table 2 shows the amount of damaged starch and the activity of endogenous amylases in flour and treated flour. When fungal α-amylase is added, the amount of damaged starch is about 5%, and the difference between treatments was not significant. When fungal α-amylase was omitted, the measurement should show the action of endogenous

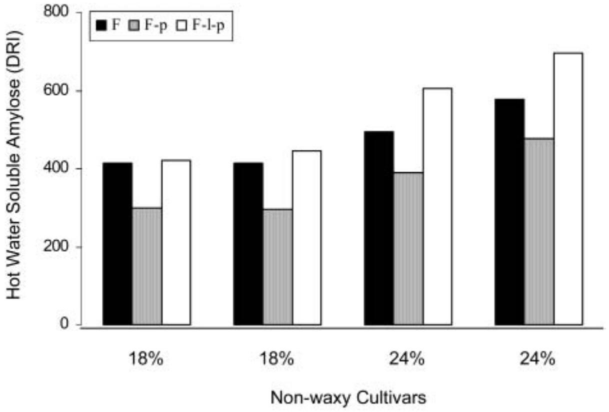

Figure 2. Amount of hot-water-soluble amylose measured by differential refractive index (DRI) from flour (F), from flour with proteins removed (F–p), and from flour with proteins and lipids removed (F–l–p), for the four varieties that contain amylose.

Table 2. Amount of Damaged Starch and Activity of Endogenous Amylases in Flour, Flour with Proteins Removed, and Flour with Proteins and Lipids Removed

	damaged starch (%)	
sample	fungal α-amylase	no fungal α-amylase
full flour	5.38	0.65
flour without proteins	5.53	0.19
flour without lipids or proteins	5.40	0.17

amylases in damaging the starch. **Table 2** shows that the activity of endogenous amylase is negligible, and furthermore, none of the treatments introduced any starch-degrading activity.

DISCUSSION

Viscosity curves are one of the most useful tools for rapidly and reproducibly assigning rices to different quality classes, but are probably underutilized because the biological and rheological contributions of polymers and their complexes to forming the curve are unknown. Studies have not been able to correlate sensory data with any viscosity parameter (*9*), indicating a need to understand how the curve is formed. In a study that tried to understand the curve, it was suggested that comparing varieties at a fixed peak height, rather than fixed concentration, would be more meaningful (*14*). Clearly, determining the concentration that gives the same peak height for many samples is inefficient and would introduce concentration as another variable. By deconstructing the flour and varying the water, we can examine the role of each component in forming the curve, and potentially understand more about how the curve could be used to predict properties of rice.

Contribution of Water to Viscosity Curves (F). The results in **Figure 1** suggest that decreasing the proportion of water leads to an increase in pasting temperature (though slight), an increase in breakdown, and a decrease in the lift-off from trough to final, consistent with findings in another study (*4*). The slight differences in pasting temperature probably reflect more the changes in the slope of the pasting curve. Further, changing the concentration of water changes the shapes of the curves, but does not change the relationships between varieties, suggesting that water affects the rheological responses to stirring of the components of the gelatinized and swollen flour, but does not accentuate biological differences between varieties.

For all six cultivars, the gel/paste at peak viscosity contains a dispersed phase of swollen granules and hydrated proteins. As the proportion of water in the sample decreases, there is greater potential for interaction between these components, and thus the viscosity increases sharply. For varieties that contain amylose, the gel/paste also contains a continuous, viscous matrix of solubilized amylose (*7*). Amylose will leach from granules after they gelatinize (*22*) suggesting that water is first bound by the amylopectin and the remainder is the solute for amylose in the continuous phase. Therefore, as the concentration of starch increases, the continuous matrix contains more amylose and less water and becomes increasingly viscous (*7*, *15*). The contribution of amylose thus could explain the exponential rate of increase in the peak viscosity with decreasing water in nonwaxy varieties compared with the linear increase observed in waxy varieties.

As the concentration of water decreases, the amount of breakdown increases for the four nonwaxy varieties (**Figure 1**; **Table 1**). The bonds linking the continuous matrix of amylose break more easily than those linking the dispersed phase of the swollen starch granules (*7*). Therefore, in constant shear, a more concentrated continuous matrix would be more susceptible to damage from shear. Studies on corn starch indicate that structural breakdown sustained during shear reduces the matrix to a viscous solution, rebuilding of the matrix from a viscous solution is slow or negligible (*7*). If increased breakdown indicates a greater degree of shearing when the concentration of water is lower, reassembling the paste during retrogradation is likely to be slower, perhaps explaining the relative decrease in final viscosity seen in **Table 1** and **Figure 1**.

In conditions of low shear stress in flour (**Figure 1a**), differences between varieties and amylose classes are large. However, decreasing the concentration of water increases the degree of shear stress, and masks many of the biological differences between varieties and shows instead rheological responses of the gel to heating and stirring. Since stirring is not generally a process in rice cooking, interpreting viscosity data might be more meaningful when shear is minimized by increasing the concentration of water.

Contribution of Proteins (F−p) to Viscosity. Figure 1 shows that proteins affect the height of the peak and the final viscosity. **Table 2** shows that removing proteins did not introduce a starch-degrading enzyme. The reduction in peak viscosity in rice when proteins are removed may reflect increased availability of water for starch since it has been shown for wheat that proteins account for about 8% of the flour but bind 40% of the water (*2*).

The results also show clearly that proteins account for differences in peak height between varieties. The effect of removing proteins was especially pronounced in one of the waxy varieties and in one of the high-amylose varieties (**Figure 1a,d**). Many storage proteins become sticky when hydrated (*16*), so differences in proteins between varieties could easily contribute to viscosity of either the dispersed or the continuous phase.

The initial rate of increase in viscosity during pasting is biphasic in flour (**Figure 1a,b**), but not when proteins are removed (**Figure 1d,e**; **Table 1**). Proteins might be responsible for this biphasic behavior since they hydrate in similar conditions to those that allow amylopectin to melt (*3*).

In most varieties, breakdown is a higher proportion of the peak when proteins are removed (**Figure 1a,d**). These results indicate that denatured proteins support the structure of the matrix and inhibit the thixotropic nature of the starch. Since the continuous matrix is the most susceptible to breakdown (*7*), perhaps the denatured proteins stabilize the continuous matrix or strengthen the links between the dispersed and continuous phases. That proteins offer some protection against breakdown is further supported by the greater lift-off observed when proteins are present (**Figure 1a,e**).

Contribution of Lipids and Proteins (F−l−p) to Viscosity. In order to avoid the effect of shear, the 2 g set (**Figure 1a,d,g**) will be discussed. Removing lipids and proteins changes the curve relative to flour, but not by damaging the starch (**Table 2**). As discussed above, removing proteins decreases viscosity (**Figure 1a,d**). When lipids are also removed, the gradient from onset to peak is shallower, the peak is broader, breakdown and final viscosity are less, and the relative viscosity is switched in the waxy and the high amylose pair (**Figure 1a,d,g**). Also the amount of HWSA increases (**Figure 2**), suggesting that the concentration of the continuous matrix increases.

The rice endosperm contains 0.5% lipids in membranes around compound starch granules (*17*, *18*), in complexes usually with smaller amylose molecules (LAM) (*19*), and in complexes with linear regions of amylopectin (LAP) (*20*). The contribution of LAP to viscosity is well illustrated in one of the waxy varieties where proteins account for most viscosity (**Figure 1a,d**). By then removing lipids in that variety (**Figure 1g**), peak viscosity is higher than it was in flour (**Table 1**), suggesting that lipids impeded hydration of the starch granules; removing lipids allowed amylopectin to contribute to viscosity.

Relative to flour, the amount of HWSA is greater without lipids or proteins, but this is not reflected in final viscosity or setback (**Figure 1a,g**). Since lipids tend to complex with smaller amylose (*21*), liberating that amylose by using methanol to remove the starch lipids would increase the amount of soluble amylose, decrease the average molecular weight of the soluble amylose fraction, and remove the opportunity for LAM complexes to form during the heating or holding stages. Further, causing more amylose molecules to leach from the swollen starch granules would decrease the integrity of the gelatinized granule in the dispersed phase. Since both the dispersed and continuous matrixes differ from those in flour, one would expect different viscosity curves and different relative viscosity between varieties. In this treatment, the architecture of the starch granule contributes more to viscosity and discriminates between varieties. However, in flour, the presence of lipids tends to mask that contribution.

CONCLUSION

The concentration of water seems to affect mostly the degree of shear. An increase in the ratio of flour and water results in increased breakdown, thus changing lift-off and the significance of final viscosity. Resistance to shear is a large factor in RVA in the conditions typically used for rice samples, and it masks much of the potentially available information about the transition from a powder to paste. This probably explains the poor correlation between RVA profile and sensory data on cooked rice (*9*) particularly since the resistance to shear depends on variety of rice. Proteins play a key role in peak and final viscosity, and also contribute to the biphasic behavior sometimes noted during the initial phases of pasting. The removal of lipids liberates amylose from LAM complexes and thus increases the amount of amylose in the continuous phase, changing its interactions and behavior. Loss of that amylose from the dispersed phase changes the integrity of the starch granules. Together, these lead to a different viscosity curve. Now that we understand more about how components of the flour determine viscosity parameters, the next step is to understand how those individual and interactive contributions define the sensory properties of rice. Then we will be able to use viscosity curves to predict accurately the sensory properties of rice.

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