

# Pasting Process in Rice Flour Using Rapid Visco Analyser Curves and First Derivatives

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## ABSTRACT

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The objective of these studies was to gain a better understanding of the pasting process in rice. We chose six different medium grain rice flour samples with amylose contents of 0.41–24.9% and protein contents of 4.89–10.65%. By using the first derivative of Rapid Visco Analyser (RVA) curves, changes in the pasting rates could be obtained. We found that samples containing low amylose contents (CM101 [CA] 0.41% amylose and 7.04% protein) exhibited a single smooth transition during pasting. Pastes from all other samples, M201 (TX), Nato (LA), Koshihikari (CA), Mercury (LA), and Nanking Sel (LA) with higher amylose contents

(10.65–24.9%) underwent multiple phase transitions and rate changes before the peak viscosity. Disruption of disulfide linkages using dithiothreitol (DTT) led to a decrease in the rate of the single pasting step observed for CM101 (CA). Rice containing larger concentrations of amylose showed an increase in the first, but a decrease in subsequent steps. Our data suggests that amylopectin and protein are mutually important in the initialization of pasting in rice. At later stages of pasting, amylose and its complexes seem to become important.

It was once believed that the physical properties of rice were controlled by amylose. More recently, work has shown that other components in rice should be considered in explaining physical characteristics. For example, the texture of rice has been linked to nonamylose components (Champagne et al 1999). Similarly, pasting properties have been linked to other grain contents. Some of the components that affect the pasting properties are protein (Teo et al 2000), phosphorous (Lin and Czuchajowska 1998), and amylopectin unit chain lengths (Silverio et al 2000). Starch, which is composed of amylose and amylopectin, can have a myriad of structural variations, such as the degree of branching, branch chain length, and helical structure. Each structural attribute can afford different degrees of interactions or complexation with other rice components such as proteins and phospholipids (Hamaker et al 1990). For instance, the helices of amylose seem to form inclusion complexes with lipids (Banks and Greenwood 1972) that play a role in rheological properties. Diversity in starch structure and composition can result in a variety of viscoelastic properties.

The component in rice with the second highest concentration is protein. Rice protein consists mainly of the storage protein, glutelin or oryzenin (Chrastil 1990). Other proteins include albumins, globulins, and prolamins. But their role in establishing rheological properties is not fully understood. Chrastil (1990) suggested that oryzenin-starch binding was one of the most important factors affecting stickiness and found a linear relationship between the binding ratios and stickiness. But a nonlinear relationship between textural properties and protein content has also been found (Champagne et al 1999).

Because the cooking step creates the physical properties necessary for the development of texture in products (Caldwell et al 2000), it is important to correlate pasting properties to composition. In this way, the physical outcome of specific rice compositions may be determined. A popular method for studying the pasting properties in grains is with a Rapid Visco Analyser (RVA). An RVA measures the viscosity as the sample is stirred, heated, and gelatinized. This results

in the leaching out of amylose molecules and breakdown of the amylopectin matrix. The exact role of granular components such as proteins and lipids bound in the starch matrix is obscure and is not always apparent in RVA curves. However, there are obvious differences in the rheological behavior of rices with different compositions, which suggests that composition-related information is hidden in viscograms.

These curves are often broad and smooth, and only drastic rate changes or phase transitions are observable. This prevents the display of details during pasting. However, differentiated viscograms have been useful in providing greater details of starch pasting (Lai and Chao 2000). In this study, we used first derivatives of viscograms for determining relationships between protein, starch, and pasting. This was accomplished through the reduction of protein disulfide bonds in rice flour and observing the viscosity rate changes that occur during pasting.

## MATERIALS AND METHODS

Six medium grain rice samples were selected from rice obtained from USDA-ARS laboratories in Beaumont, TX, and New Orleans, LA. These samples have amylose contents of 0.41–24.90%. The protein content was 4.89–10.65%. Both values were determined and discussed in previously (Barton et al 1998). Milling and other procedures were also discussed.

### RVA Measurements

A Newport Scientific Super 3 type Rapid Visco Analyzer (RVA) (Foss North America, Inc., Eden Prairie, MN) was used for RVA studies. Rice samples were run in duplicate. First 25 mL of distilled water was added directly to a metal RVA canister. Then  $3.00 \pm 0.01$  g of rice flour was weighed, added to water, and immediately measured by the RVA. These measurements were made with the standard Newport Scientific rice profile that ramps from 50 to  $95^\circ\text{C} \pm 1$  in 0 to 5 min with cooling to  $50^\circ\text{C}$  over 7–12.5 min. Each profile was initiated by a 10 sec, 960 rpm paddle speed for mixing, followed by a 160 rpm paddle speed for the remainder of data collection. Data was taken at a rate of one data point per second. First derivatives (Savitsky-Golay over 11 data points) of viscograms were done by exporting data into Grams 32 v. 5.21 (Galactic Industries, Salem, NH).

### Dithiothreitol (DTT) Studies

Dithiothreitol (DTT) 99% pure was purchased from ACROS (Fairlawn, NJ). A 5 mM solution of DTT was prepared by adding dry sample to the appropriate amount of water. The pH of water and the DTT solution was measured using a pH meter (model 611, Orion Research, Boston, MA). Instead of deionized distilled

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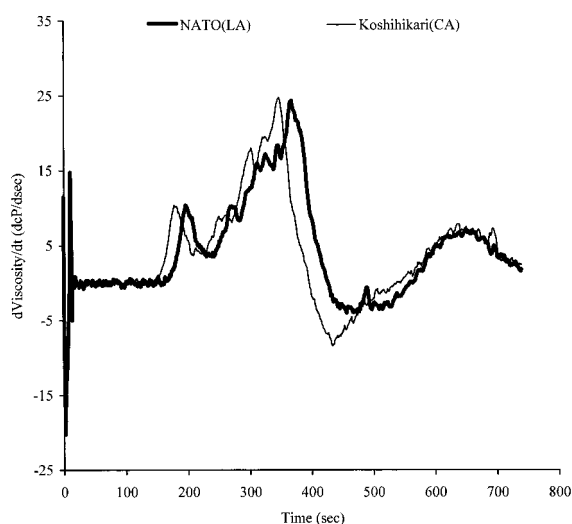
water, 25 mL of the 5 mM DTT solution was added directly to the RVA canister before each measurement. The rice flour was then added to the DTT solution and measured by RVA.

## RESULTS AND DISCUSSION

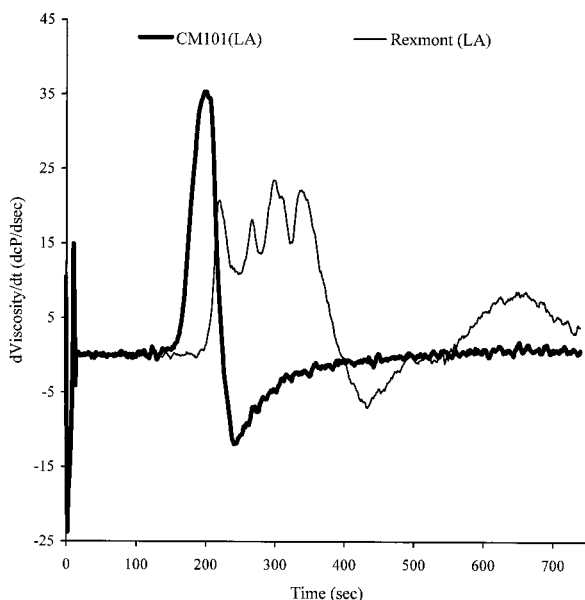
### First Derivative Studies

Different rice samples exhibit various pasting temperatures, times and viscosity changes. However, these details are often hidden in normal RVA curves. The typical RVA output is a smooth curve with viscosity measured in Rapid Visco Units (RVU) or centiPoise (cP) (y-axis) as a function of time (sec) (x-axis). First derivative viscosgrams represent the rate of viscosity change (dcP or dRVU) with respect to time (dt). Hence, many of the phase changes (rate changes) that occur during RVA measurements become observable in differentiated viscosgrams. This approach can afford a clearer explanation of rice pasting with respect to composition.

We selected six medium grain rice flours with varying amylose contents for discussion of the pasting process. A first derivative plot of these viscosgrams shows distinct differences in the pattern



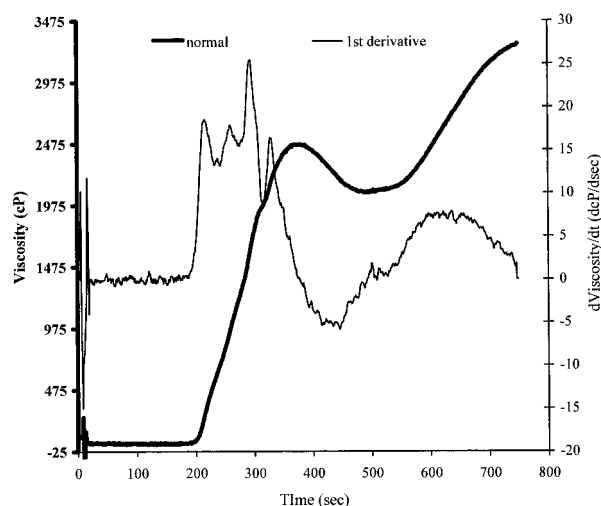
**Fig. 1.** First derivative viscosgram of NATO (LA) (8.74% protein and 15.75% amylose) and Koshihikari (CA) (4.89% protein and 15.95% amylose) rice flour.



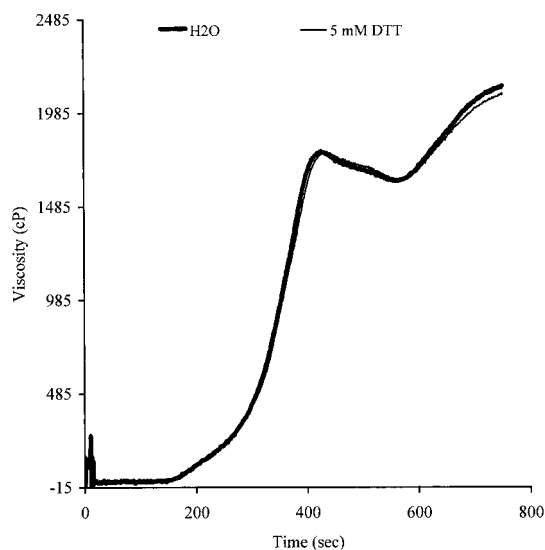
**Fig. 2.** First derivative viscosgram of CM101 (LA) (7.04 protein and 0.41% amylose) and Rexmont (LA) (7.98% protein and 23.70% amylose) rice flour.

of slope changes before reaching the peak viscosity. Samples with different protein concentrations (4.89 and 8.74%) but similar amylose concentrations (15.95 and 15.75%) showed little difference in the number of major slope changes that occurred (Fig. 1). However, there were differences for the times at which slopes changes appeared. The number of slope changes was drastically different for samples at similar protein concentrations (7.04 and 7.98%) but different amylose percentages (23.7 and 0.41%) (Fig. 2). These differences suggest that the rate of pasting is related to protein, while the complexity of the pasting process relies on starch composition.

An overlay of the normal and first derivative RVA curves of Nanking Sel (LA), high amylose rice, shows the most dramatic rate changes occur after reaching the gelatinization temperature but before the breakdown period (Fig. 3). Viscosity intensity changes are slow outside of this region. On the other hand, first derivative viscosgrams for low amylose rice, CM101 (CA), produces a single smooth curve with the exception of the initial noise from paddle rate changes (960–160 rpm) between 0 and 10 sec (Fig. 2). The large rate change and narrow peak width implies that the rate of water uptake and granular swelling is not inhibited by limited solubility. In this case, water uptake seems to occur continuously near  $79 \pm 1^\circ\text{C}$ . If the pasting process is more complex for low amylose rices, the ability to visualize details will be limited by the maximum RVA sampling rate.



**Fig. 3.** Normal and first derivative viscosgrams of Nanking Sel (LA) rice flour (24.9% amylose and 9.65% protein) in water. Bold y-axis (left) corresponds to the normal curve.

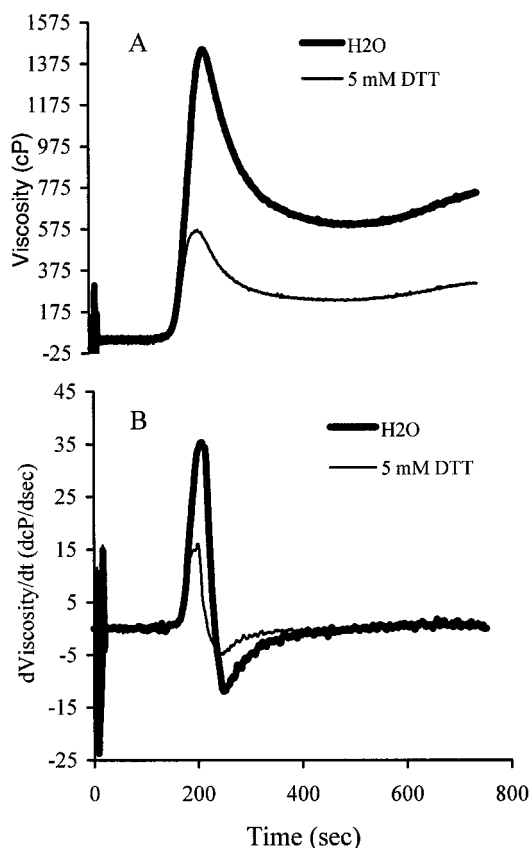


**Fig. 4.** Isolated rice starch in water and treated with 5 mM DTT.

Rice samples with a higher amylose content including M201 (TX), Nato (LA), Koshihikari (CA), Mercury (LA), and Nanking Sel (LA) exhibited a series of phase changes before reaching peak viscosity (Fig. 3). We will not attempt to assign chemical or structural origins of these stages. However, there is an overlap of the first peaks of all samples studied. This peak seems to originate from amylopectin as indicated in the CM101 (CA) plot and may serve as a marker for amylopectin and its importance to starch granules. This idea is plausible because amylopectin dominates granular structure and physical properties in starch (Zobel 1988). Subsequent transitions for the higher amylose samples seem to relate to less soluble components such as crystallites of longer chain lengths that need greater heat energy for solubilization (Jane et al 1999). Each transition may be linked to specific complexes that dissociate at different rates with the least soluble fraction leaching out of the granule last. This thought implies that complexes such as amylose-lipid would correspond to the later transitions seen in the pasting process. Our data is consistent with the description of pasting being a complex series of events leading ultimately to the complete diorganization of the granular structure (Caldwell et al 2000). Higher amylose rice undergoes slower and more complex pasting than low amylose rice.

### DTT Studies

We determined the general role of disulfide-containing protein on the pasting process. This was accomplished by reducing disulfide bonds in protein using DTT as demonstrated by Hamaker and Griffin (1990). We focused on the effect protein has on pasting, as indicated by the differentiated viscograms of rice flour. A well-defined picture of the effect protein concentration has on pasting rates may be gained with a sample set containing a wide variety of protein contents for long, medium, and short grain rice with any



**Fig. 5.** A, Viscograms of CM101 (CA) rice flour (0.41% amylose and 7.04% protein) in water and in 5 mM DTT. B, First derivative viscograms of CM101 (CA) under same conditions.

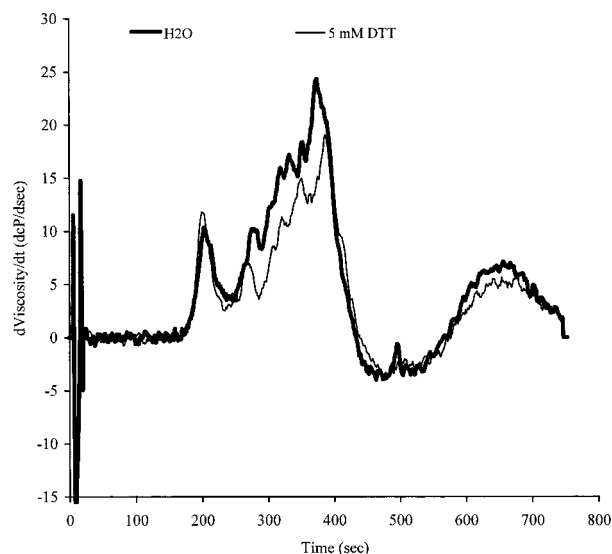
potential interfering components being at constant concentration. Such samples were not available at the time of these studies.

As a control, commercial rice starch was used to ensure that DTT did not affect starch (Fig. 4). There were no significant differences in starch viscograms with and without DTT. Treatments with DTT did not affect the pasting of other components in rice (Hamaker and Griffin 1990). The pH of 5mM DTT (6.0) and water (6.1) was measured to determine whether viscosity changes could be attributed to pH. Because there was only a small difference in pH, major effects on protein conformation and starch-protein interactions seem unlikely but should not be ruled out.

In rice flours, differences occurred with each of the traditional RVA parameters after the addition of reducing agent (Table I). Most of these values showed a decrease with DTT treatment with the exception of breakdown for Nanking Sel (LA) and M201 (TX), and setback for CM101 (CA). Peak times occurred earlier for the untreated Koshihikari (CA) and Mercury (LA) rices. Although there are obvious changes, there does not appear to be a well-defined relationship between protein content and RVA parameters. Because DTT reduces disulfide bonds, differences in RVA viscosities and rate values are likely due to the various amounts of disulfide bonds present in each rice type. The quantity of disulfide bonds may or may not be proportional to the total protein concentration. In the absence of such a relationship, links between RVA parameter changes and total protein may not be established.

The first derivative plot of CM101 (CA) showed a decrease in pasting rate and pasting time-temperature with disulfide bond cleavage (Fig. 5). All other rice flours, M201 (TX), Nato (LA), Koshihikari (CA), Mercury (LA), and Nanking Sel (LA), had increased pasting rates, decreases in pasting times-temperatures for the first step with reducing agent present (Fig. 6). This is an opposite effect than is seen in samples containing low amylose. These differences in DTT effects may depend on the routes taken during pasting. When the content of amylopectin is high, water absorption is accomplished more readily. At higher amylose concentrations, there may be an increased propensity to form complexes making a multistaged pasting process more likely. In all samples exhibiting this complex pasting process, decreases in viscosity and rates were exhibited in the second stage. With the exception of Nanking Sel (LA), all of the transitions beyond the second stage occurred at slower rates and lower viscosities with DTT.

All of the curves in this study overlap with the single observable pasting step in CM101 (CA), which is mostly composed of amylopectin (0.41% amylose). This first stage in pasting is most affected



**Fig. 6.** First derivative viscogram of Nato (LA) (15.75% amylose and 8.74% protein) rice flour in water and in 5 mM DTT.

**TABLE I**  
**Differences in RVA Values (cp) for Rice Flours With and Without Dithiothreitol (DTT) Treatment**

Cultivar	% Protein	% Amylose	Peak	Trough	Breakdown	Final	Setback	Peak Time (sec)
Koshihikari (CA)	4.89	15.9	32.5	21.8	10.8	40.9	8.44	-0.12
CM101 (CA)	7.04	0.41	66.4	27.2	39.2	32.7	-33.8	0.17
Mercury (LA)	8.45	14.1	9.3	35.1	4.25	53.5	14.2	-0.06
Nato (LA)	8.74	15.8	45.7	41.6	4.14	51.3	5.61	0.01
Nanking Sel (LA)	9.65	24.9	17.8	26.6	-8.92	39.3	21.5	0.29
M201 (TX)	10.7	10.7	21.7	32.1	-10.3	47.3	25.6	0.02

<sup>a</sup> Differences in parameters represent means of duplicate determinations.

by DTT, and therefore, protein. With amylopectin as the first starch component solubilized in the pasting process, this information may indicate the joint importance of protein and amylopectin in the structure and function. Several additional explanations may exist for the peak overlap including the dissociation of unique protein-starch complexes (e.g., oryzenin-starch). Although protein seems to be centrally important, starch structure seems to control the number of pasting phase changes that occurs in high amylose rice.

It has been suggested that granular swelling is not the only factor controlling the increase in viscosity (Miller et al 1973). Even after granules have collapsed, the viscosity continues to rise. One explanation is that the concentration of granular exudate is the primary factor responsible for starch paste consistency. The slower rise to peak viscosity observed with DTT present may be understood as inaccessibility of water to granular components due to protein. Chrastil (1990) discussed the fact that interactions are permitted between starch granules and proteins with cooking. These interactions may be more prevalent and easily seen in the first derivative viscograms of high amylose rice. Disulfide bond cleavage may decrease protein folding, which can expose binding sites for binding other rice constituents. Thus, the rate at which granular components leach out can be reduced. Similarly, nonprotein complexes may be formed. For example, lipid-amylose complexation increases with pasting time (Kaur and Singh 2000).

In high amylose rice, the first pasting stage occurs at a slightly greater rate when treated with DTT. Differences between the initial and later stages of pasting may relate to the time needed for granular breakdown and freeing of bound protein to form new complexes. The increase in pasting rate at the first step indicates that protein has a structural significance, which is coincident with the pasting of amylopectin. Only when granular constituents such as amylose and lipids, etc., are solubilized later in the pasting process can protein form new complexes and limit granular exudation.

### CONCLUSIONS

The use of differentiation is helpful in extracting pasting information from viscograms. Rate and phase changes that are unobvious in nondifferentiated viscograms were found. Our data shows that protein and starch structure play an important role in pasting. It is possible that protein complexes with amylopectin and granular structures and causes an inhibition of the initial pasting of rice at low amylopectin concentrations. When proteins are treated with DTT, complexes can dissociate increasing the rate of water absorption. This is a plausible explanation given that amylopectin is thought to be responsible for the initial uptake of water. The rate enhancement of the first stage of pasting with disulfide bond disruption with low amylopectin is concurrent with the rate decrease of CM101 (CA), a high amylopectin sample. Assuming that a simple phase transition occurs with CM101 (CA), there may be major differences in starch-protein interactions for low and high amylopectin samples. The exact role that proteins have in pasting may come through the elucidation of protein structures. To date the crystal structure of the major protein in rice, oryzenin, has not

been reported. Protein and starch structural information should reveal details about protein binding capacities and behaviors that may be important to rheological properties. This information may benefit the rice industry for making genetic modifications and exploiting value-added qualities.

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