Effect of Addition of Zein and Gliadin on the Rheological Properties of Amylopectin Starch with Low-to-Intermediate Moisture[1]

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ABSTRACT

The effect of protein-starch interaction on the rheological properties of 98% amylopectin starch with intermediate-to-low (20–40%) moisture content and in the temperature range of 100–150°C was examined using zein and gliadin proteins. Protein-starch interactions resulted in increased viscosity once a threshold temperature was exceeded. The threshold temperature is dependent on the moisture content of the system and the nature of the protein. The lower the moisture content is, the higher the threshold temperature is. Gliadin + amioca had a lower threshold temperature compared to zein + amioca.

All cereals flours (corn flour, oat flour, wheat flour, rice flour, etc.) contain different levels of starches (amylose and amylopectin), proteins (albumins, globulins, prolamins, and glutelins), and lipids (polar and nonpolar). The resulting rheological properties of cooked cereal flours strongly depend on order-disorder transitions in each chemical constituent (starch, protein, and chemical transformations) as well as secondary and tertiary interactions among them. Temperature and water content are also important factors affecting chemical and physical interactions among the constituents. In this article we examine the effect of the addition of protein on starch rheological properties in limited-moisture environments.

Starches, when heated in excess water, undergo swelling and subsequent dissolution. Models of starch gelatinization have been proposed by Remsen and Clark (1978) and Gomez and Aguilera (1984). Wang et al (1989) showed that 14 molecules of water per molecule of anhydrous glucose were necessary for starch to undergo complete gelatinization. Differential scanning calorimetry data suggest that starches with a limited quantity of moisture have peak transition temperatures that increase with a decrease in moisture content (Breslauer et al 1988, Wang et al 1989).

Starch granules undergo changes when subjected to shear in the presence of limited moisture (less than 14 molecules per mole of anhydrous glucose) and higher temperatures. Some of these changes are not seen in cases of excess water and absence of shear. These changes can be characterized as losses of birefringence and crystallinity (Anderson et al 1970, Bhattacharya and Hanna 1987) and reductions in molecular size of both amylose and amylopectin (Colonna et al 1984, Davidson et al 1984, Klingler et al 1986, Wen and Wasserman, 1990). Mechanical stresses result in the reduction of molecular size of the amylopectin fraction (Basedow et al 1979, Davidson 1983). Wang et al (1991) established that when starch is subjected to shear stresses, the conversion (gelatinization and/or melting) temperature is reduced based on the intensity of the applied shear stress.

Transformations in proteins are equally complex. Changes in protein due to heat processing can be separated into four different types (Bender 1978).

The first type of change, which requires only mild heat, is an alteration of tertiary structure (denaturation). The physical and chemical properties of proteins are changed: globular protein can suffer changes in solubility, viscosity, osmotic properties, and electrophoretic mobility, among others that accompany the unfolding of long chains, with the liberation of reactive groups such as amino, hydroxyl, carboxyl, and sulfhydryl groups.

The second type of change is caused by mild heat in the presence of reducing substances and results in linkage between the end (ϵ) amino group of lysine with a reducing group. This is Maillard or nonenzymatic browning.

In the third type of change, more severe heating reduces the availability of other amino acids, as well as lysine, and can occur in the absence of reducing substances. Cysteine is relatively sensitive and can be converted into compounds such as methyl mercaptan, dimethyl sulfide, and dimethyl disulfide at temperatures of 115°C. There is usually a fall in digestibility as well. Reactions can take

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place within proteins themselves between free amino groups of lysine and arginine and free acid groups of aspartic and glutamic acids or with amide groups such as asparagine and glutamine. Amino acids can react with sulfur groups, particularly cysteine and to a lesser extent methionine and the imidazole ring of histidine.

The fourth change is caused by excessive heat applied to the outside of roasted foods, which leads to the destruction of amino acids by complete decomposition or by racemization and formation of cross-linkages forming polyamino acids. Temperatures of 180–300°C, such as are involved in roasting coffee, meat, and fish and in baking biscuits, have these effects.

Thermal transition temperatures increase with decreasing moisture content (Mitchell and Areas 1991). A concentrated solution of protein melt is formed by the proteins dissolving into a superheated aqueous phase over a range of temperature (Ledward and Mitchell 1988).

Proteins that undergo reversible thermal denaturation under one set of conditions can undergo irreversible thermal denaturation in a slightly different set of conditions. Thermal denaturation often results in the formation of aggregate molecules that have first undergone reversible denaturation. The rate of appearance of irreversibility is generally accelerated by an increase in protein concentration, showing that the reaction is higher than first order. The mechanism for formation of the aggregates includes formation of intermolecular cross-links by disulfide interchange (Warner and Levy 1958). Bonds or interactions that are responsible for maintaining the secondary and tertiary structure of native proteins (hydrogen bonds, hydrophobic interactions, ionic interactions, and disulfide linkages) do not appear to survive the high temperatures and shear forces encountered during shear processes such as extrusion even if such associations are formed during the heating process. Additionally, secondary bonding interactions are disrupted when the temperature is raised further and therefore will not interfere with the formation of melt or concentrated solution.

D'Appolonia and Shelton (1984) suggested that starch, the major component of wheat flour, is unique when compared to starches from different sources in its ability to combine with wheat gluten to produce a loaf of bread.

Bushuk et al (1984) have shown that glucose and polar lipids interact with specific proteins of gluten to form large aggregates. They postulated that such aggregates of gluten contribute significantly to the rheological properties of gluten and to breadmaking. It is well documented that gluten washed from wheat-flour dough contains about 10% carbohydrate (Zawistowska et al 1985). Carbohydrate associated with glutenin protein forms a stable complex and cannot be disrupted by various solvents or physical agents. Carbohydrates are postulated to be associated with glutenins through hydrogen bonds or hydrophilic hydrophobic interactions but not by covalent bonds. Rice endosperm protein interacts strongly with amylopectin (B. R. Hamaker and P. H. Ball, *personal communication*, 1990). The interaction does not involve disulfide bonding. Kato et al (1990) prepared protein-dextran conjugate by first freeze-drying an ovalbumin-dextran mixture (1:5) and then storing it at 60°C (65% relative humidity) for three weeks. Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, they showed that ovalbumin was covalently attached to dextran and formed the conjugate. Ovalbumin-dextran conjugate showed a high weight-average molecular weight in the range of 200,000, determined using low-angle light scattering, compared to 60,000–90,000 for dextran.

Chedid and Kokini (1992) have also shown that the addition of protein (glutenin, glutelin, gliadin, zein) to corn starches resulted in synergistic increases in viscosity, which suggests significant interactions between starch and protein. The interaction occurred once the temperature was elevated above the gelatinization temperature of starch.

The objective of this research was to examine the effect of the addition of protein on the rheological properties of starch in a limited-moisture environment. Specifically, a high-amylopectin starch (98% amylopectin) was studied in the presence of the proteins zein and gliadin, using rheological measurements with a capillary rheometer in the temperature range of 100–150°C and a moisture range of 20–40% on wet basis.

The second objective of this work was to examine the effect of the method of mixing on the rheological properties of starch-protein mixtures. The three methods of mixing examined were freeze-drying starch-protein mixtures, dissolving protein in 70% ethanol and vacuum-drying the starch-protein, and dry mixing.

TABLE I
Length and Diameter of Rheometer Capillaries

Length (in.)	Diameter	Length to Diameter
1.0	0.06	16.67
2.0	0.05	40.00
3.0	0.05	60.00
4.0	0.03	133.33

MATERIALS AND METHODS

Sample Preparation

Amioca (98% amylopectin) (lot AG 4208, National Starch Company, Bridgewater, NJ) was used in this study. Zein and gliadin were obtained from Sigma Chemical Co. (lots 84F-0155 and 15F-0182, respectively, St. Louis, MO).

Initial moisture content was determined by AACC (1983) method 44-15A and was 10.1% for amioca. The starch protein ratio was kept constant at 12 parts of protein to 88 parts of starch on a dry basis. This ratio was selected because it represents the average ratio of starch to protein commonly found in flours. Starch-protein mixtures were prepared by freeze-drying, mixing in 70% ethanol and vacuum-drying, and dry mixing.

Freeze-dry mixing. A mixture of 88 g of starch plus 12 g of protein on dry basis (zein or gliadin) was suspended in 100 ml of water at room temperature. This suspension was then freeze-dried for about two days. Moisture content of the freeze-dried sample was determined.

Vacuum-dry mixing. A predetermined amount of zein or gliadin was dissolved in 70% ethanol in the ratio 1:12.5 under constant stirring. Amioca starch was then suspended in the protein solution to generate a starch-protein ratio of 88:12. This suspension was vacuum-dried at 60°C, under 5 psi, for about 48–72 hr until the sample was dried to 8% moisture. The dried material lumps were broken by mortar and pestle and sieved through a 20-mesh screen.

Dry mixing. Dry ingredients were blended together. Water in the required amount was added to the starch-protein powder and mixed thoroughly. The mix was then sieved through a 20-mesh sieve to break the lumps, if any, and was then allowed to sit overnight to hydrate and equilibrate at 4°C. It was brought to ambient temperature before loading in the barrel of the capillary rheometer.

Rheological Measurements

A capillary rheometer (model 3211 series 290, Instron, Canton, MA) was used for the rheological measurement of amioca, protein (zein and gliadin), and amioca-protein for samples with moisture content of 40, 30, and 20% at high temperatures, that is, at and above 100°C. The capillary used had length-to-diameter ratios as shown in Table I.

The rheometer barrel, which was maintained at 60°C, was filled with an equilibrated sample. The plunger was allowed to descend at a very low speed. As the material started coming out at the capillary end, the heater was switched on to raise the temperature of the barrel. As temperatures of 100, 120, and 150°C were reached, measurements of force at different shear rates from 2.6 to 1,000 sec^{-1} were taken. The measurements were performed in triplicate, and the data presented are averages of three readings. The Rabinowitch-Mooney correction was used to correct for shear rates of non-Newtonian fluids; however, end correction was not performed. The power-law model $\tau_\omega = m^*(\gamma)^n$ for non-Newtonian

fluids (where τ_ω is the wall shear stress and γ is the shear rate) was applied to obtain a consistency index m and a flow-behavior index n. These parameters were used to calculate viscosity at the shear rates given by the other capillaries. The average of experimental viscosity with one capillary and the calculated viscosity with the other capillary at identical shear rates were taken to plot viscosity vs. shear rates on a log-log scale.

RESULTS AND DISCUSSION

Effect of Mixing Method on Rheological Properties

Figure 1 shows the effect of the mixing method on rheological properties of the gliadin amioca mixture at 120°C with 40% and 100°C with 20% moisture contents, respectively. In Figure 1A amioca + gliadin shows nearly identical viscosity for vacuum-dried, freeze-dried, and dry-mixed material. All of these methods produce mixtures that, when heated to 100°C with 20% moisture (Fig. 1B), had slightly different viscosities. Freeze-dried samples showed higher viscosity than dry-mixed and vacuum-dried samples. This indicated that the method of mixing did not significantly change the mode of interaction between amioca and zein or gliadin at the temperatures and moisture contents of the experiments. The most significant of these three methods is the case where gliadin is dissolved in 70% ethanol and mixed with amioca in that environment. Ethanol (70%) is a good solvent for gliadin and therefore completely deaggregates the protein. This allows greater molecular contact between the protein and starch, generating conditions that favor interaction. Clearly this method of mixing did not result in any substantial increase or decrease in viscosity, showing that dry mixing or freeze-dried mixing were equivalent as mixing methods to dissolving the gliadin in ethanol and then adding amioca and vacuum-drying. This result led us to adopt dry mixing because it is the most convenient to use.

Effect of Added Zein and Gliadin on the Rheological Properties of Amioca

Rheological measurements for amioca, protein, and amioca + protein showed that viscosity was a function of shear rate, moisture content, temperature, and type of protein used. Figure 2 shows the viscosity of amioca, zein, and amioca + zein with 30% moisture at 100°C. The viscosity of amioca was the largest of all shear rates studied, that of zein was the smallest, and that of amioca + zein was intermediate between those of amioca and zein alone. The measured viscosities were consistent with the molecular size of each cereal biopolymer. Amylopectin weight-average molecular weight is reported to be above 50–500 $\times 10^6$ (Zobel 1988), although that of reduced zein is reported to be in the range of 22,000 and 24,000 (Landry and Guyon 1984). Low molecular weight zein gave lower viscosity, as expected. It is interesting to observe that at this temperature there were no

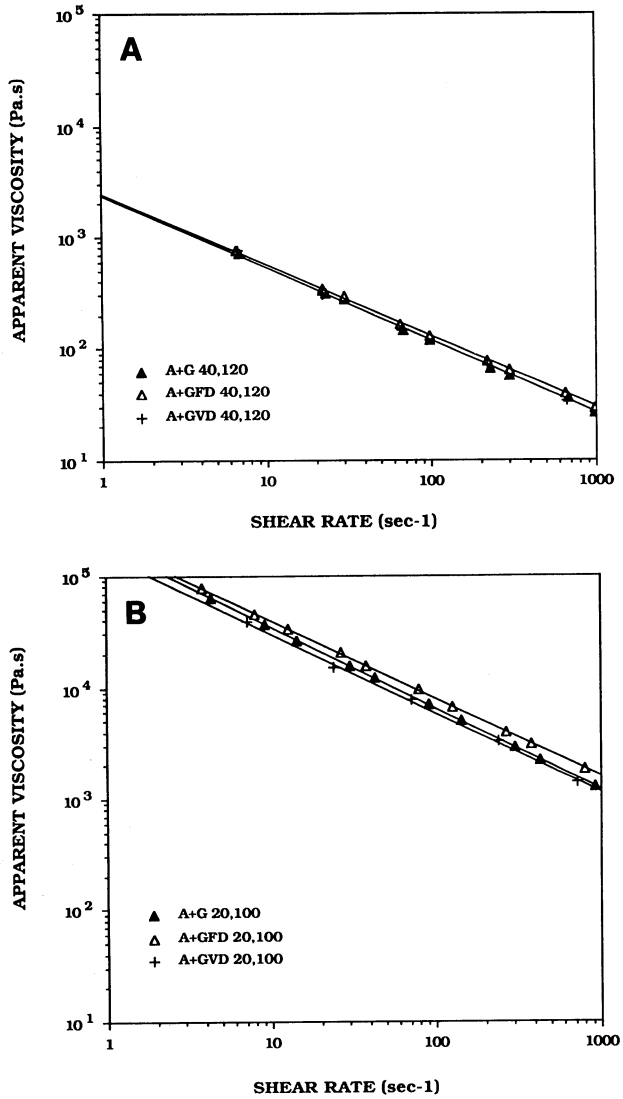


Fig. 1. Effect of mixing method on amioca + gliadin. **A,** moisture 40% 120°C; **B,** moisture 20% 100°C. A+G = amioca + gliadin, FD = freeze-drying, VD = vacuum-drying.

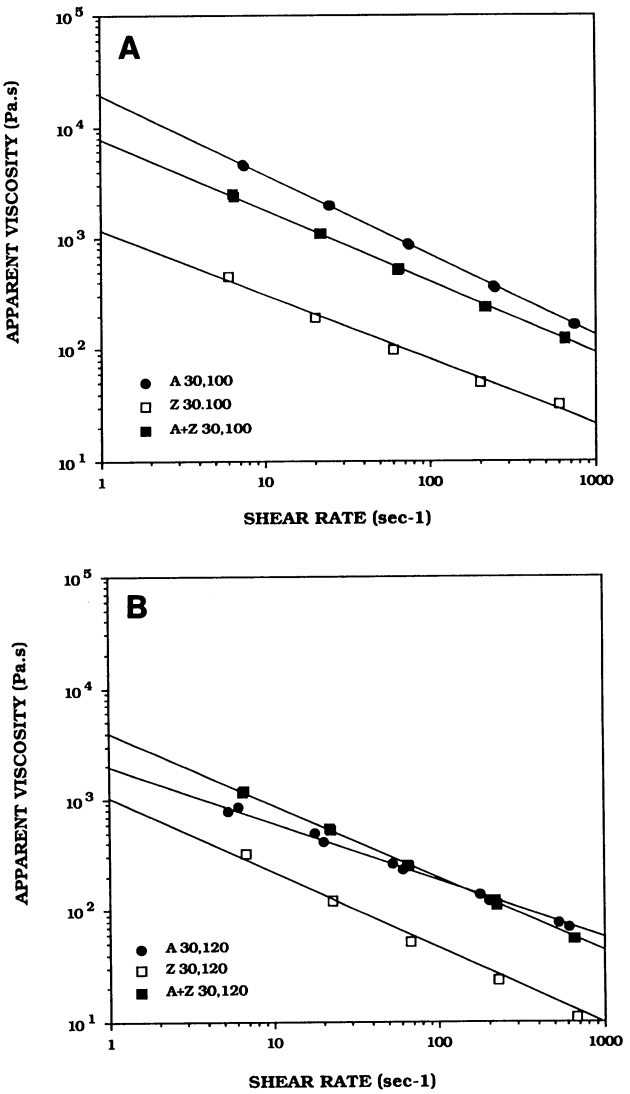


Fig. 2. Viscosity of amioca (A), zein (Z), and amioca + zein. **A,** moisture 30% 100°C; **B,** moisture 30% 120°C.

increases in the viscosity of the mixtures of zein and amylopectin. This clearly suggests that zein acts as a diluent, reducing the viscosity of amylopectin. When the temperature was increased to 120°C, as shown in Figure 2B, the viscosity of the amioca + zein system was higher than both amioca and zein alone at lower shear rates. This increase in viscosity suggests that zein and amylopectin are interacting with each other, resulting in associations between the two molecules and thereby increasing the viscosity of the mixture. The temperature at which such associations were observed appears to be a function of moisture content. For example, amioca + zein mixtures showed an increase in viscosity at 100°C, but only when moisture contents were in the range of 55–75% (Chedid 1989).

Figure 3 shows the viscosity of amioca + gliadin mixtures with a 30% moisture content at 100° C. Here the viscosity of the mixture was larger than that of either amioca or gliadin alone. The temperature required for gliadin to interact with amylopectin seemed to be lower than for zein at 30% moisture content. Because gliadin is a relatively less hydrophobic protein compared to zein, it may interact with amylopectin more readily, resulting in increased viscosity. Moreover, gliadin hydrates more easily than zein. Stabilizing forces in the tertiary and quaternary protein structure are known to work through hydrogen bonding, hydrophobic interactions, and electrostatic interactions. Hydrophobic interactions, being endothermic in nature, require more thermal energy input. Therefore, an increase in the temperature of the system from 100 to 120°C might have been necessary to allow zein molecules to interact with starch in the presence of 30% moisture.

Figures 4–6 compare the viscosity of amioca with that of amioca + zein and amioca + gliadin in the temperature range of 100–150°C and moisture range of 20–40%. At 20% moisture content and 100°C, both amioca + zein and amioca + gliadin viscosities were lower than viscosity of amioca alone. At this temperature and moisture content no increase in viscosity was observed (Fig. 4A). On the other hand, at 120°C (Fig. 4B) amioca + gliadin viscosity was almost identical to that of amioca alone, which in turn was higher than that of amioca + zein. Therefore, at 120°C an increase in viscosity in gliadin + amioca is observed. This might be due to interactions between gliadin and amylopectin that result in a loosely held network. The origins of this network structure are not fully understood, but a comparison of data at lower temperatures suggests that there is a threshold temperature for this behavior to occur, and the threshold temperature is also a function of moisture content as well. At 150°C (Fig. 4C) amioca + gliadin had a viscosity very much higher than amioca. At the same time amioca + zein had a lower viscosity than amioca.

At 30% moisture content (Fig. 5) the threshold temperature for interaction shifted to a lower temperature as the temperature for conversion (gelatinization and/or melting) decreased with the increase in moisture content. For example, at 100°C amioca + gliadin had a viscosity higher than amioca alone, although amioca + zein had a lower viscosity. When we compare this result for gliadin + amioca at 30% moisture content to the previous observa-

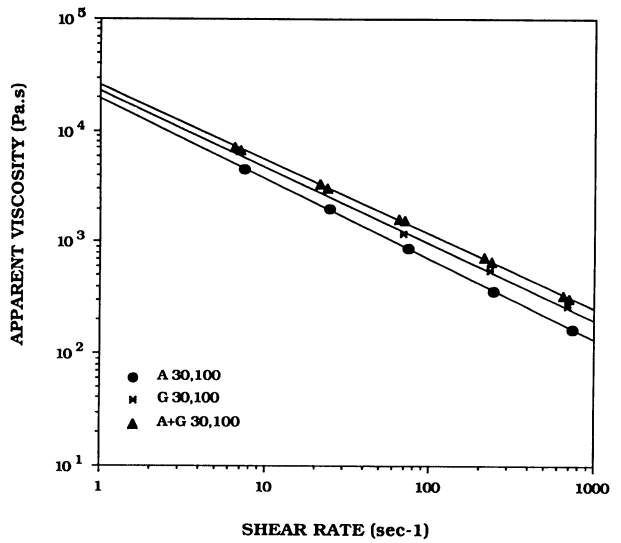


Fig. 3. Viscosity of amioca (A), gliadin (G), and amioca + gliadin, moisture 30% 100°C.

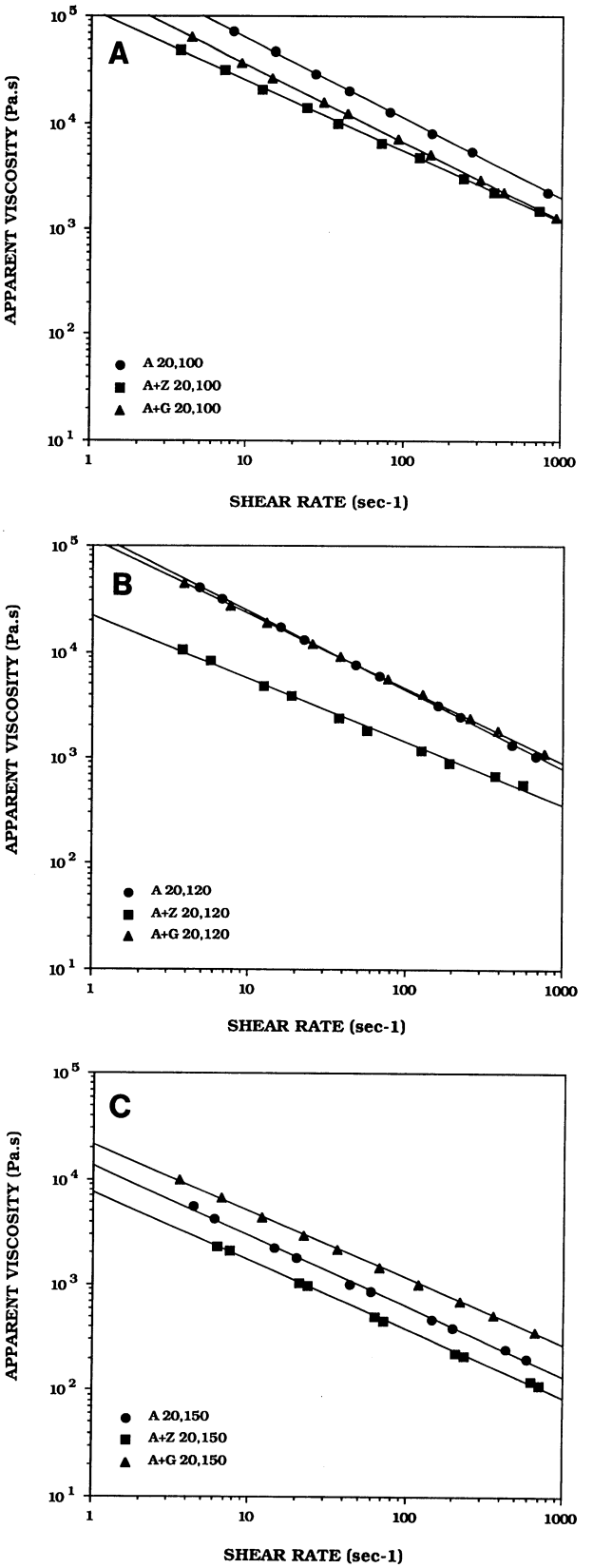


Fig. 4. Effect of zein (Z) and gliadin (G) on viscosity of amioca (A). **A,** moisture 20% 100°C; **B,** moisture 20% 120°C; **C,** moisture 20% 150°C.

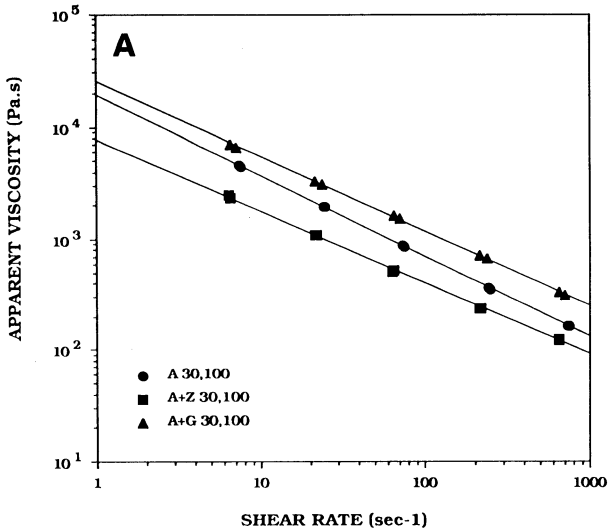
tion at 20% moisture content, we see that the higher moisture content has facilitated the development of a so-called network formation and thereby increased in viscosity. At 100 and 120°C amioca + gliadin had higher viscosities relative to amioca alone. Amioca + zein had a lower viscosity than amioca at 100°C, but as the temperature increased to 120°C, amioca + zein showed higher viscosity at lower shear rates than amioca alone. The second significant observation is that amioca + gliadin had a much higher viscosity than amioca + zein. Again, with 20% moisture content zein + amioca did not show significant increases in viscosity at 150°C, whereas with 30% moisture viscosity increases at 120°C.

At 40% moisture content, the interaction further shifted to lower temperatures (Fig. 6). This suggests that the melting and gelatinization of starch plays a very important role in terms of facilitating increases in viscosity between starch and protein. The fact that both starch gelatinization and protein denaturation temperature shift to lower values suggests that these two transformations are at the origin of the observed increases. At this moisture content and at all temperatures studied, starch-protein had a higher viscosity compared to starch alone.

It can be envisioned that there exists a threshold temperature for proteins to enter into association with amylopectin that is dependent on moisture content and should be a function of the hydrophobicity or hydrophilicity of the protein because zein had a higher threshold temperature than gliadin. Consequently, the conclusion that is emerging from this research is that the higher the hydrophobicity of the protein, the higher the threshold temperature. The threshold temperature for gliadin to interact with amioca starch was lower than that for zein. This may be due to the fact that gliadin is less hydrophobic than zein.

As the temperature rises much above the conversion temperature of starch, the effect of starch-protein interactions may be balanced by the disruption of hydrogen bonds and hydrophobic bonds and also by the breakdown of starch molecules, resulting in a drop in viscosity.

Measuring the viscosity of the starch-protein system above 160°C was not possible because the viscosity of the material was extremely low and the shear stress required could not be read without significant error, and the flashing of moisture as well as nonenzymatic browning reactions would cause the capillary to clog.



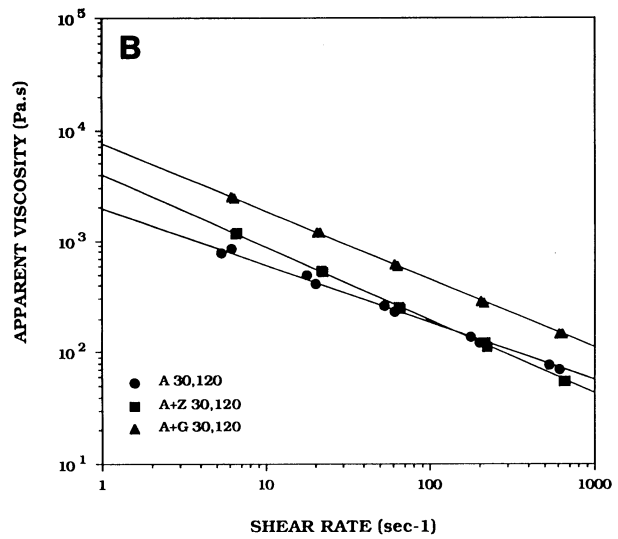


Fig. 5. Effect of zein (Z) and gliadin (G) on viscosity of amioca (A). **A,** moisture 30% 100°C; **B,** moisture 30% 120°C.

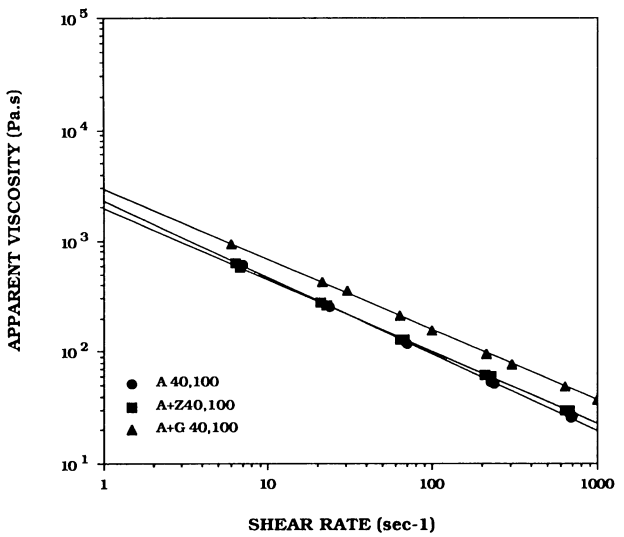


Fig. 6. Effect of zein (Z) and gliadin (G) on viscosity of amioca (A): moisture 40% 100°C.

TABLE II
Power-Law Parameters: Consistency Index *m* and Flow Behavior Index *n* for Amioca, Amioca + Zein, and Amioca + Gliadin

Condition		Amioca		Amioca + Zein		Amioca + Gliadin	
Moisture (%)	Temperature	*m**1,000[a]	*n*	*m**1,000[a]	*n*	*m**1,000[a]	*n*
20	100 C	341.430	0.250	111.330	0.340	185.300	0.272
	120 C	130.680	0.260	21.719	0.406	113.410	0.300
	150 C	135.600	0.330	7.396	0.353	20.847	0.374
30	100 C	19.307	0.280	7.611	0.364	25.269	0.333
	120 C	1.973	0.487	3.907	0.351	7.569	0.389
	150 C	1.236	0.410	2.613	0.261	2.183	0.430
40	100 C	2.273	0.312	1.972	0.357	2.939	0.367
	120 C	1.535	0.317	0.751	0.372	2.337	0.354
	150 C	0.640	0.302	0.639	0.362	1.421	0.335

[a] m = Pa(sec)^n.

Booth et al (1980) and Schofield et al (1983) have reported the effect of temperature on solubility of gluten proteins. They observed that the loss of gliadin solubility in 50% propanol up to 70°C was minimal and decreased proportionally up to 100°C. The solubility could be reversed by adding the disulfide reducing agent dithioerythritol, suggesting that the formation of S-S bonds may be responsible for aggregation of gliadin and glutenin in general at high temperatures. Thus, although hydrophobic interactions in protein may be important in the association of starch-protein depending upon moisture and temperature of the system, at high moisture content disulfide bonding may play a dominant role in aggregation and network formation of the protein itself, thereby further promoting increases in viscosity.

Table II shows the consistency index (m) and flow behavior index (n) for amioca, and amioca + zein, and amioca + gliadin at low moisture contents and high temperatures. The consistency index decreased with the increase in temperature and moisture content of the system. The flow behavior index, on the other hand, increased with the increase in temperature at lower moisture, and at higher moisture content it did not change significantly. The smaller the flow behavior index, the more non-Newtonian the behavior is. The increase in the consistency index of the starch + zein and starch + gliadin system can be explained by the formation of networks among the protein molecules that entrap starch molecules or granules in the network. The covalent (disulfide) and/or noncovalent association of these networks is dependent on moisture content, temperature, the hydrophilic nature of the protein, and the conversion temperature of the starch.

ACKNOWLEDGMENT

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